

pTet MODULE: LAB LOGBOOK WEEKLY SUMMARY

Week 1 (06/06 - 10/06)

1. Transformation

- Promoter (pSB1C3_BB_a_R0040)
- Double Terminator (pSB1C3_BB_a_B1006)
- Constitutive Promoter (J61002_BB_a_J23101)
- TetR (pSB1C3_BB_a_C0040)

2. Miniprep

- TetR (pSB1C3_BB_a_C0040)
- Double Terminator (pSB1C3_BB_a_B1006)
- Promoter (pSB1C3_BB_a_R0040)

3. Restriction Digestion

- pSB1C3_BB_a_R0040_E0240 (pTet + GFP in pSB1C3)
- pSB3K3_BB_a_R0040_E0240 (pTet + GFP in pSB3K3)

Restriction Digestion of pSB1C3- BB_a_R0040 and pSB3K3-BB_a_E0240

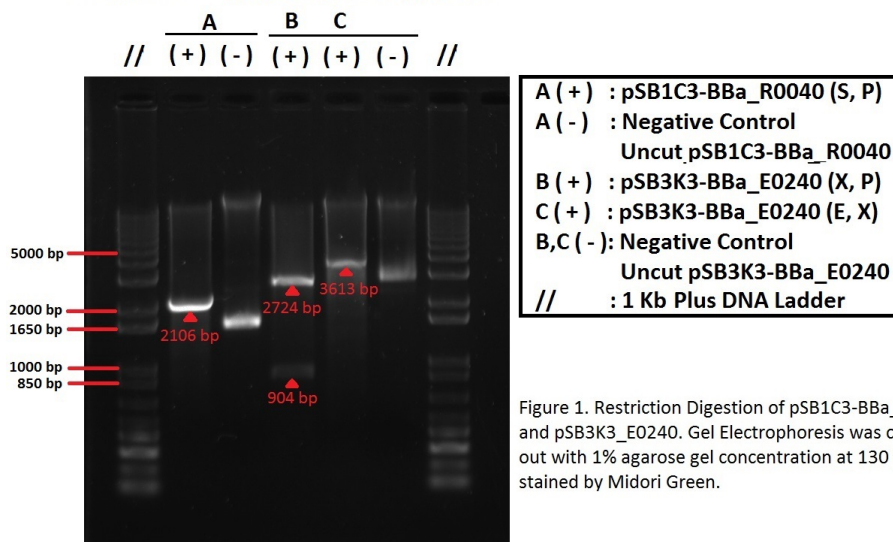
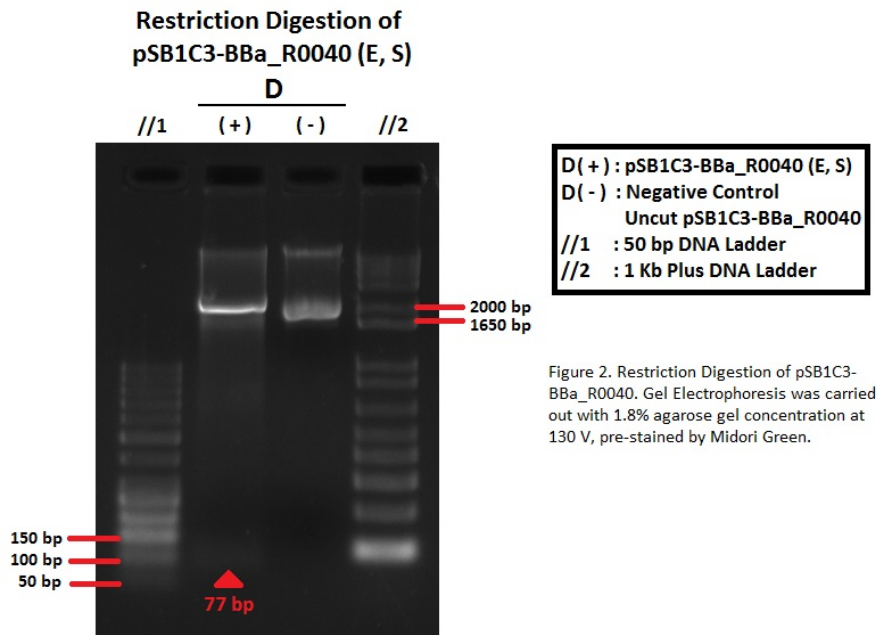


Figure 1. Restriction Digestion of pSB1C3-BBa_R0040 and pSB3K3_E0240. Gel Electrophoresis was carried out with 1% agarose gel concentration at 130 V, pre-stained by Midori Green.



4. Transformation of pSB1C3-BBa_B0034-C0040

5. Phusion PCR of

- BBa_B0030-C0040 (RBS-TetR) with B0030-C0040 FW and VR primers
- BBa_B0032-C0040 (RBS-TetR) with B0032-C0040 FW and VR primers

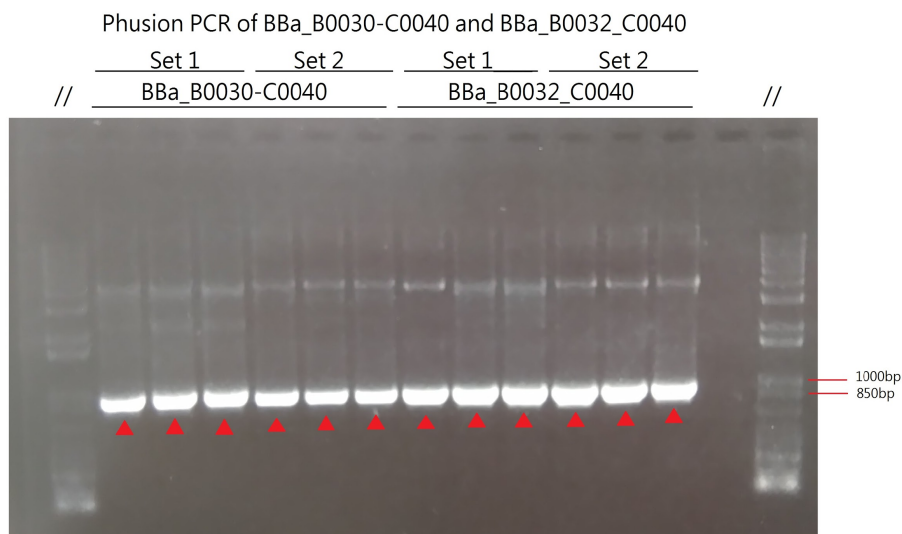


Figure . Phusion PCR of BBa_B0030-C0040 and BBa_B0032-C0040
Gel electrophoresis was carried out with 1% agarose gel concentration at 130V, pre-stained by Midori Green. The DNA sample underwent Phusion PCR was compared to 6 µl of 1 kb plus DNA ladder.

Week 2 (13/06-18/06)

1. Colony PCR of:

- pSB1C3-BBa_R0040-E0240
- pSB3K3-BBa_R0040-E0240

Colony PCR of pSB1C3-BBa_R0040-E0240 and pSB3K3-BBa_R0040-E0240

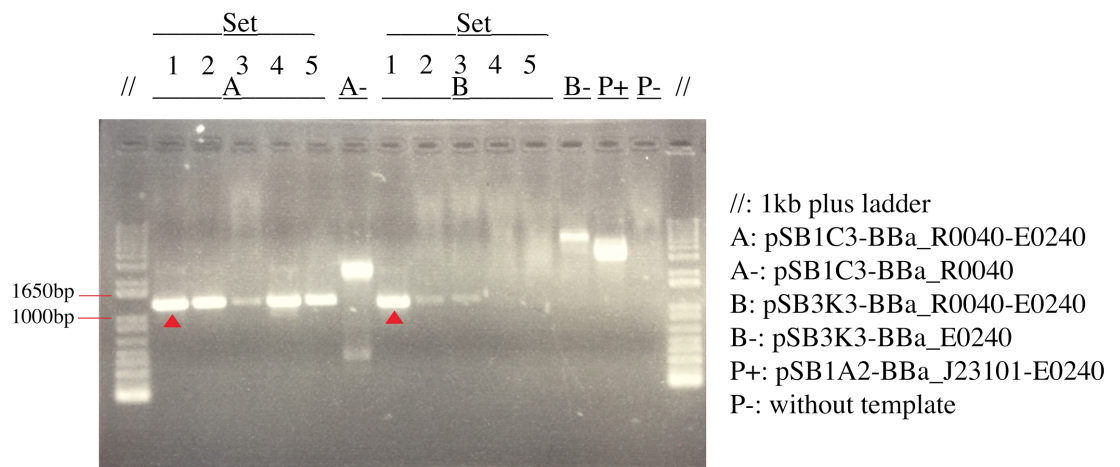


Figure . Colony PCR of pSB1C3-BBa_R0040-E0240 and pSB3K3-BBa_R0040-E0240
Gel electrophoresis was carried out at 1% agarose concentration at 130V/m, in-gel-stained by Midori Green. The template sample underwent colony PCR was compared to 6µl 1 kb plus DNA ladder.

2. Ligation of BBa_R0040_E0240 with pSB3K3 backbone

3. Restriction Check of:

- pSB3K3-BBa_R0040_E0240
- BBa_R0040_E0240

Restriction Check of pSB3K3-BBa_R0040_E0240

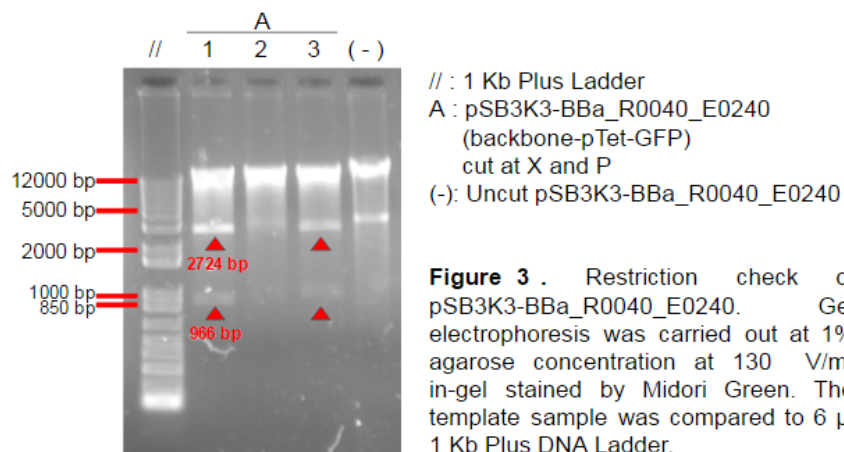


Figure 3 . Restriction check of pSB3K3-BBa_R0040_E0240. Gel electrophoresis was carried out at 1% agarose concentration at 130 V/m, in-gel stained by Midori Green. The template sample was compared to 6 µl 1 Kb Plus DNA Ladder.

4. Restriction Check of:

- pSB1C3-BBa_R0040_E0240
- pSB3K3-BBa_E0240

Restriction digestion of pSB1C3-BBa_R0040-E0240 and pSB3K3-BBa_E0240

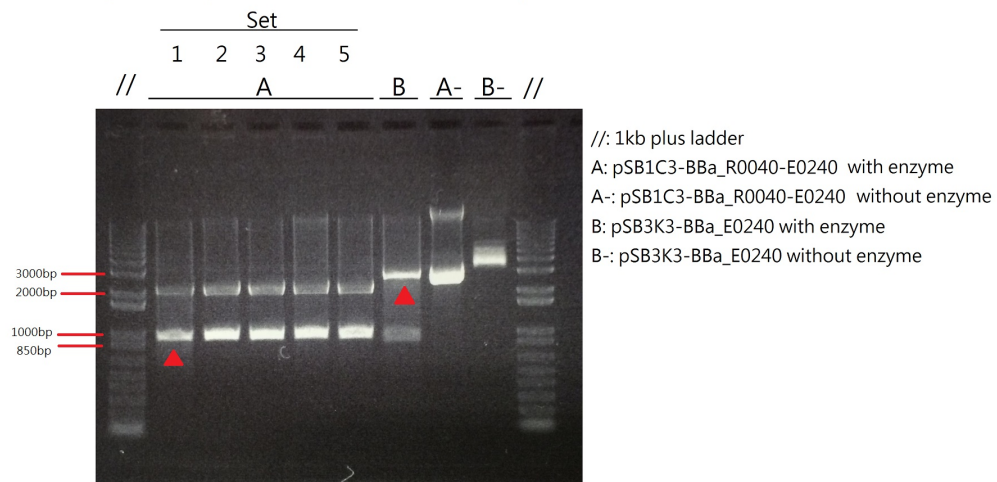


Figure . Restriction digestion of pSB1C3-BBa_R0040-E0240 and pSB3K3-BBa_E0240
Gel electrophoresis was carried out at 1% agarose concentration at 130V/m, in-gel-stained by Midori Green. The plasmid sample underwent digestion was compared to 6µl 1kb plus DNA ladder.

5. Colony PCR of pSB3K3-BBa_R0040_E0240 with VF2 and VR primers

Week 3 (20/06 - 24/06)

1. Sequencing of:

- pSB1C3-BBa_B0030-C0040
- pSB1C3-BBa_B0032-C0040

2. Ligation of J61002-BBa_J23101 with pSB1C3-BBa_B0032-C0040

3. Transformation of pSB1C3-BBa_S03518 (BBa_B0034-C0040)

4. Colony PCR with R0040 FW and VR primers

- pSB1C3-BBa_B0030-C0040
- pSB1C3-BBa_B0032-C0040
- pSB1C3-BBa_B0034-C0040

Colony PCR of pSB1C3-BBa_B0030-C0040 and pSB1C3-BBa_B0032-C0040

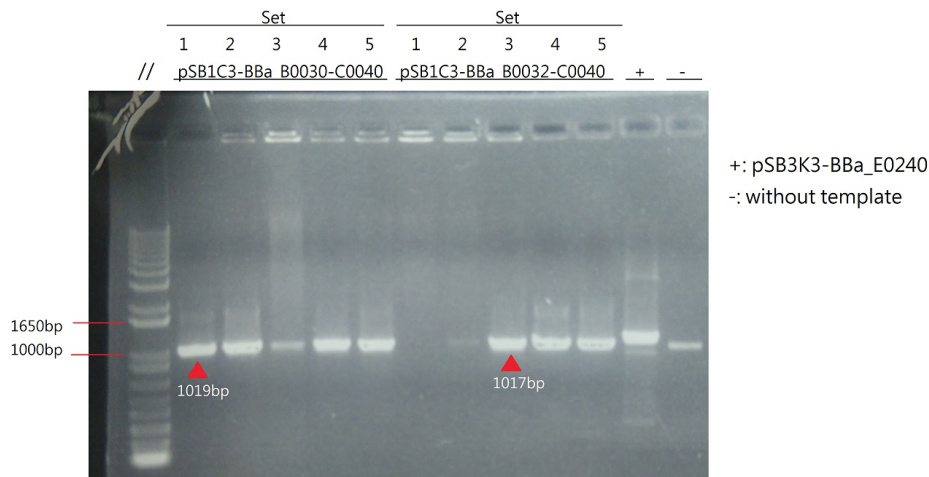
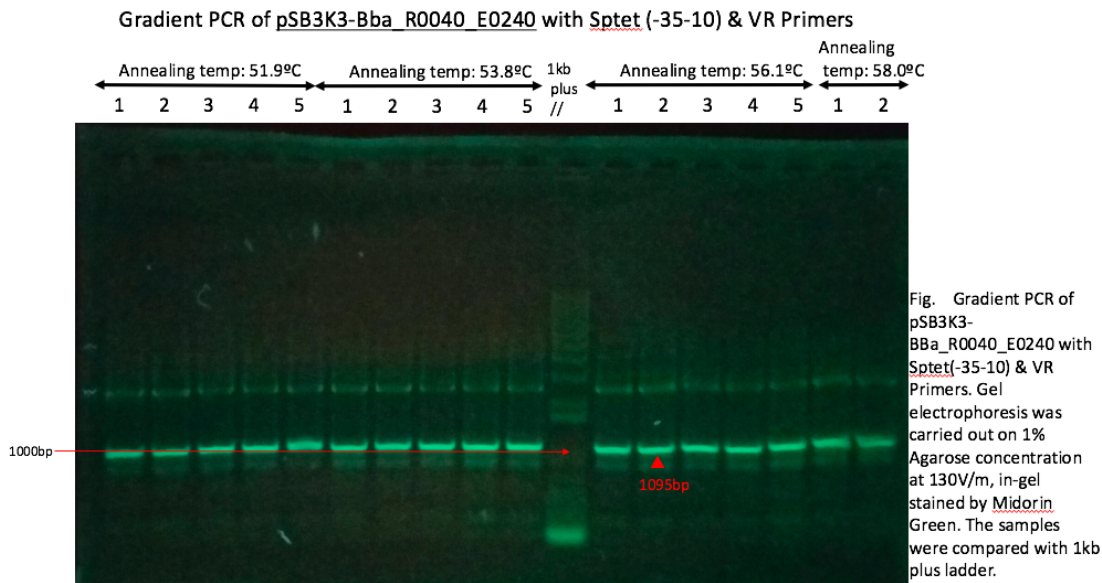


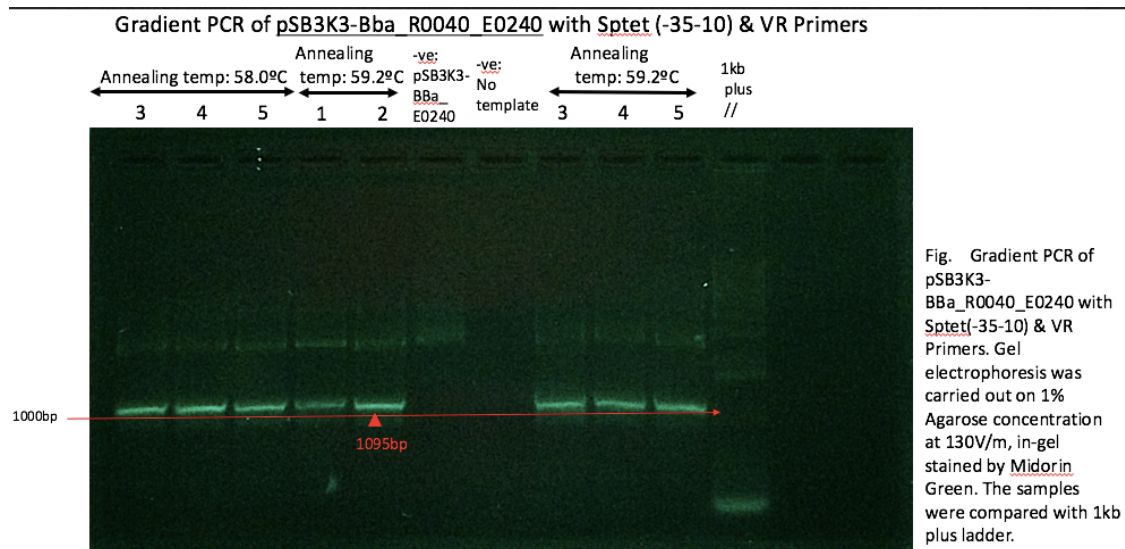
Figure . Colony PCR of pSB1C3-BBa_B0030-C0040 and pSB1C3-BBa_B0032-C0040
Gel electrophoresis was carried at 1% agarose concentration at 130V/m, in-gel-stained by Midori Green. The plasmid sample underwent Colony PCR was compared to 6µl 1kb plus ladder.

5. Miniprep

- pSB1C3-BBa_B0034-C0040

6. Gradient colony PCR of pSB3K3-BBa_R0040_E0240 with R0040 FW and VR primers.





7. Streaked pSB3K3-BBa_R0040-E0240 plates for characterisation

8. Inoculation of pSB3K3-BBa_R0040-E0240 colonies

9. First attempt of pSB3K3-BBa_R0040-E0240 characterization

10. Restriction Check and Restriction Digestion of:

- pSB1C3-BBa_B0030-C0040 with XbaI and PstI
- pSB1C3-BBa_B0032-C0040 with XbaI and PstI
- pSB1C3-BBa_B0034-C0040 with XbaI and PstI

Restriction Check of pSB1C3-BBa_B0030-C0040 and pSB1C3-BBa_B0032-C0040

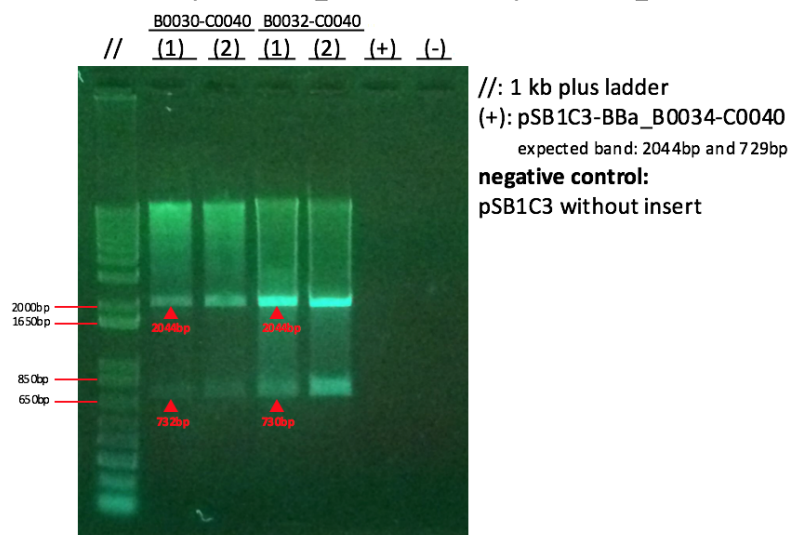


Figure . Restriction check of pSB1C3-BBa_B0030_C0040 and pSB1C3-BBa_B0032_C0040
Gel electrophoresis was carried out at 1% agarose concentration at 130V/m, in-gel-stained by Midori Green. The plasmid sample underwent digestion (2 hours; 37°C) was compared to 6µl 1kb plus DNA ladder.

11. Gel extraction and purification of pSB1C3-BBa_B0032-C0040 (X,P)

12. Ligation of J61002-BBa_J23101 (S,P) and pSB1C3-BBa_B0032-C0040 (X,P)

13. Transformation of pSB1A2-BBa_J23101-B0032-C0040

14. Sequencing of:

- pSB1C3-BBa_B0030-C0040
- pSB1C3-BBa_B0032-C0040

Week 4 (27/06 - 30/06)

1. Characterisation of pSB3K3-BBa_R0040-E0240

2. Colony PCR of pSB1A2-BBa_J23101-B0032-C0040 with VF2 and VR primers

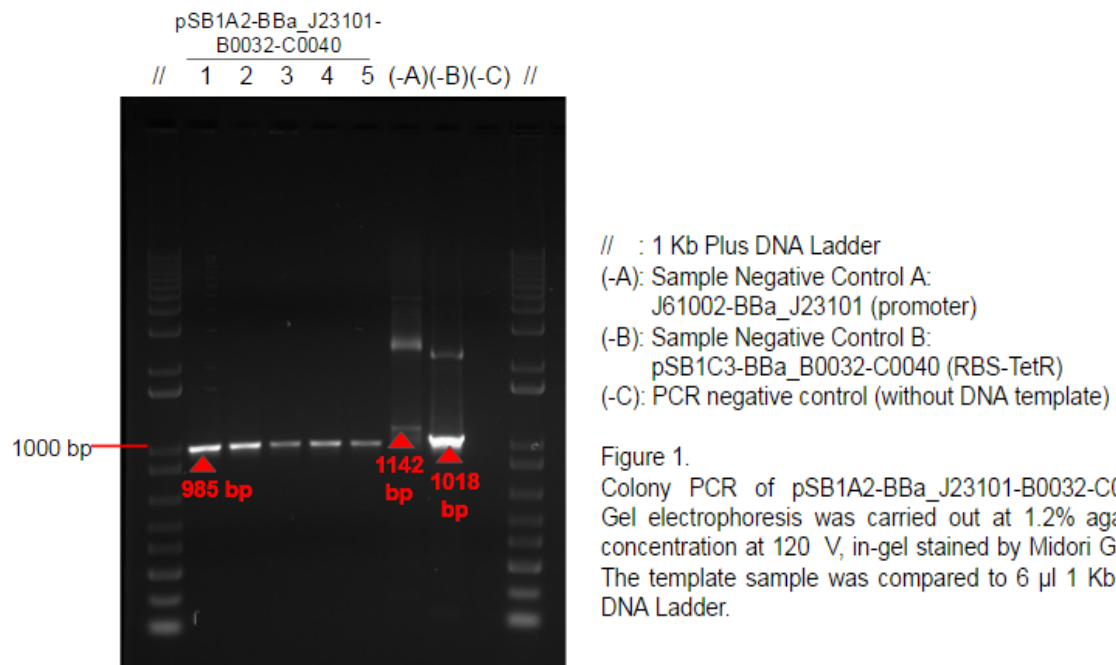
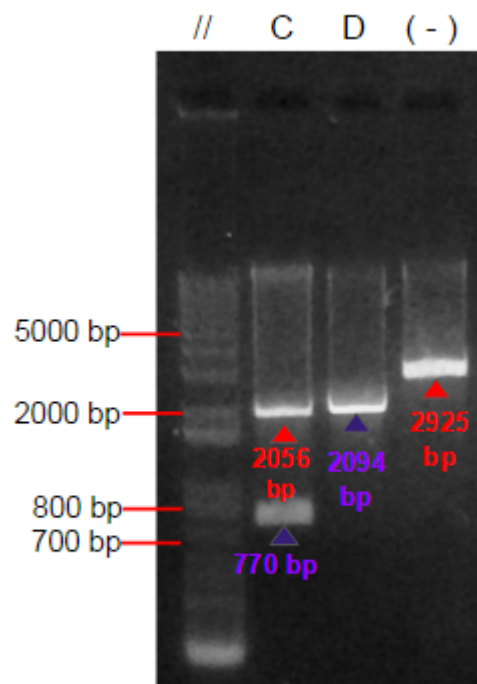


Figure 1.
Colony PCR of pSB1A2-BBa_J23101-B0032-C0040.
Gel electrophoresis was carried out at 120 V, in-gel stained by Midori Green.
The template sample was compared to 6 µl 1 Kb Plus DNA Ladder.

3. Restriction Digestion of:

- pSB1A2-BBa_J23101-B0032-C0040 with SpeI and PstI-HF
- pSB1C3-BBa_B1006 with XbaI and PstI-HF



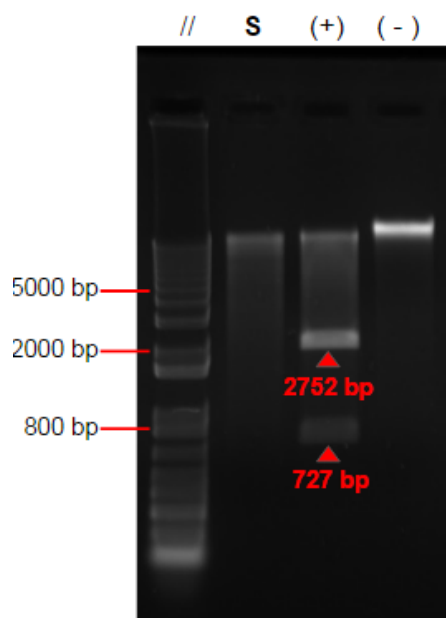
// : 1 Kb Plus DNA Ladder
C : pSB1A2-BBa_J23101-B0032-C0040 (promoter-weak RBS-*TetR*)
 cut at E and S
D : pSB1C3-BBa_B1006 (Terminator)
 cut at E and X
 (-): J61002-BBa_J23101 cut at E and S

Figure 2.
 Restriction Digestion of pSB1C3-BBa_J23101-B0032-C0040-B1006.
 Gel electrophoresis was carried out at 1% agarose concentration at
 130 V, in-gel stained by Midori Green. The template sample was
 compared to 6 μ l 1 Kb Plus DNA Ladder.

4. Ligation of pSB1C3-BBa_B1006 and pSB1C3-BBa_J23101-B0032-C0040

5. Transformation of pSB1C3-BBa_J23101-B0032-C0040-B1006

6. Restriction check of pSB1C3-BBa_S03518



// : 1 Kb Plus DNA Ladder
S : pSB1C3-BBa_S03518 cut at E and S
 (B0034-C0040) (Strong RBS-*TetR*)
 (+): pSB1C3-BBa_B0032-C0040 cut at E and S
 (-) : Uncut pSB1C3-BBa_B0032-C0040

Figure 3. Restriction Check of pSB1C3-BBa_S03518. Gel
 electrophoresis was carried out at 1% agarose concentration
 at 130 V/m, in-gel stained by Midori Green. The template
 sample was compared to 6 μ l 1 Kb Plus DNA Ladder.

The sample showed no band.

Expected band size :
S : 726 bp and 2753 bp

**>> Re-inoculated, and transformation was repeated again
 however from a different kit plate.**

Week 5 (04/07 - 08/07)

1.Characterization of pSB3K3-BBa_R0040-E0240

2. Colony PCR of pSB1C3-BBa_J23101-B0032-C0040-B1006 with VF2 and VR primers

Colony PCR of pSB1C3-BBa_J23101-B0032-C0040-B1006

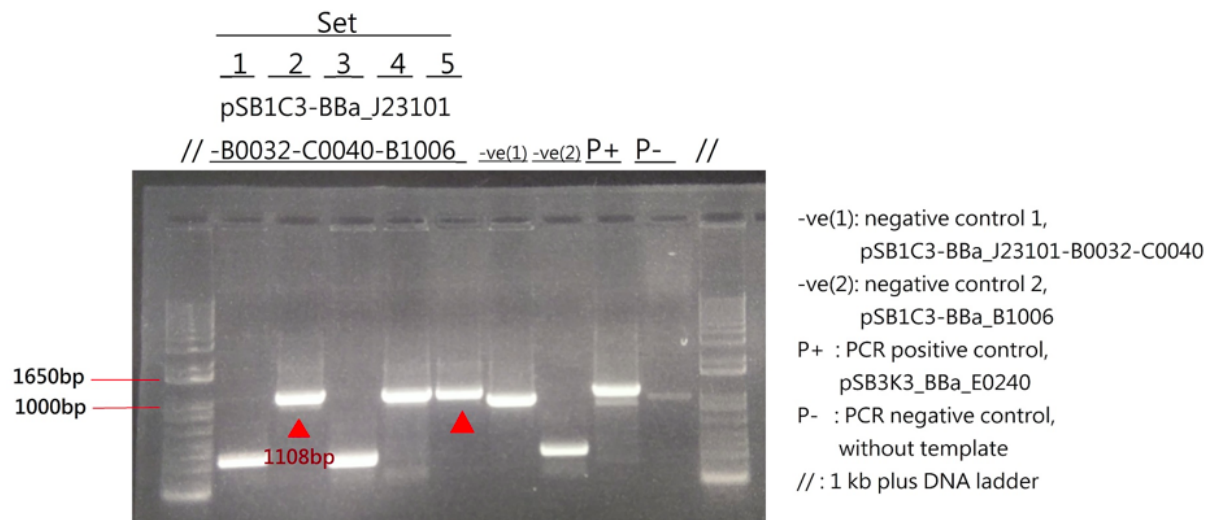


Figure . Colony PCR of pSB1C3-BBa_J23101-B0032-C0040-B1006

Gel Electrophoresis was ran at 1% agarose concentration at , in-gel stained by Midori Green. The Colony PCR products were compared to 6μl of 1kb plus DNA ladder.

3. Restriction Check and Digestion of pSB1C3-BBa_J23101-B0032-C0040-B1006

Restriction Digestion of pSB3K3-BBa_J23101-B0032-C0040-B1006

& pSB3K3-BBa_R0040-E0240

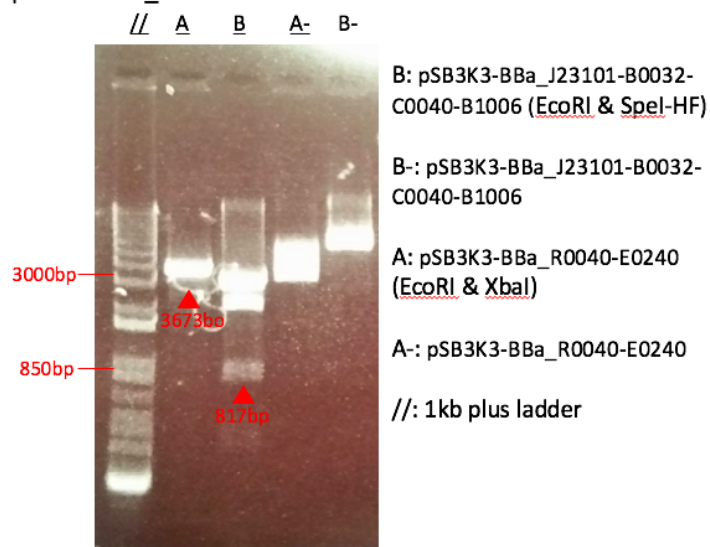


Figure . Restriction Digestion of pSB3K3-BBa_B0032-C0012 & pSB3K3-BBa_E0240

Gel Electrophoresis was ran at 1% agarose concentration at V/cm, in-gel stained by Midori Green. The Colony PCR products were compared to 6μl of 1kb plus DNA ladder.

4. Restriction Digestion of pSB3K3-BBa_R0040-E0240

5. Ligation of pSB1C3-BBa_J23101-B0032-C0040-B1006 with pSB3K3-BBa_R0040-E0240

6. Transformation of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240

7. Transformation of pSB1C3-BBa_S03518

8. Inoculation of pSB1C3-BBa_S03518

9. Colony PCR of pSB3K3-BBa_J32101-B0032-C0040-B1006-R0040-E0240

Colony PCR for pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240

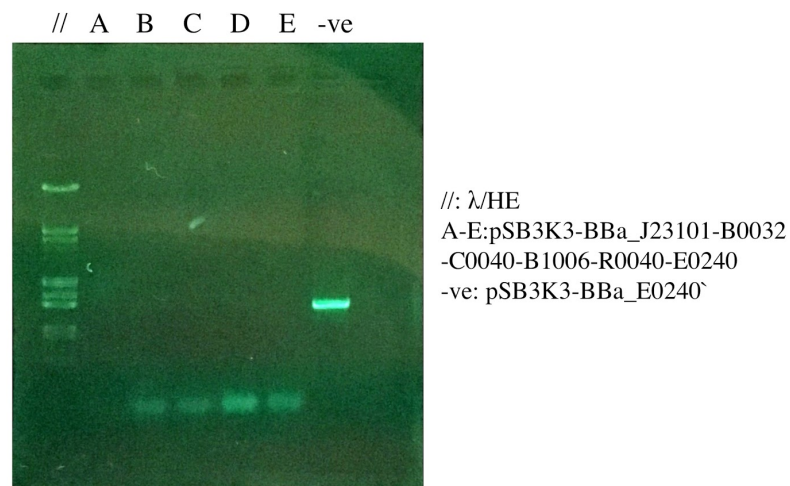


Figure . Colony PCR for pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240
Gel electrophoresis was carried at 1% agarose concentration at 130V, in-gel stained by Midori Green. The plasmid sample underwent Colony PCR was compared to 6 μ L Lambda DNA/EcoRI+HindIII ladder.

Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240 (Construct B)

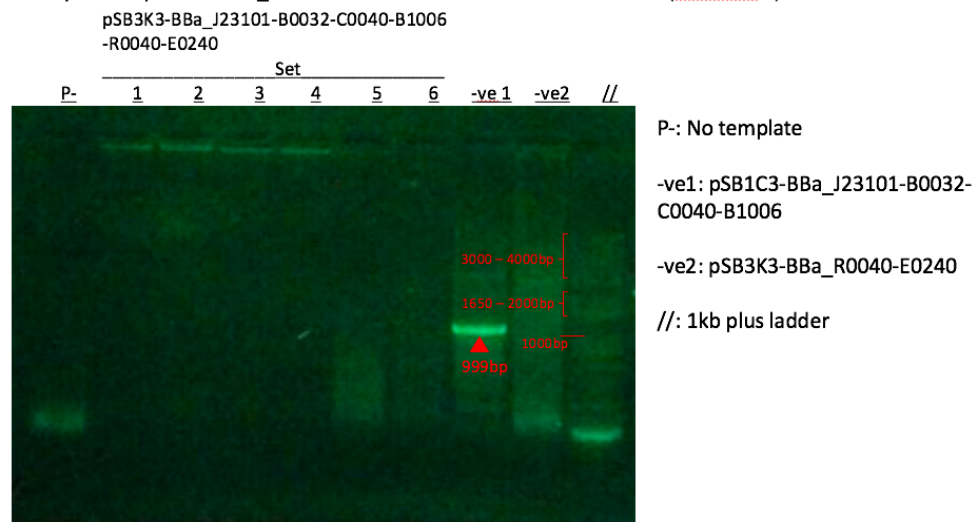


Figure . Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240
Gel Electrophoresis was ran at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The Colony PCR products were compared to 8 μ L of 1kb plus DNA ladder.

10. Restriction Digestion of

- pSB3K3-BBa_B0032-C0012
- pSB3K3-BBa_E0240

Restriction Digestion of pSB3K3-BBa_B0032-C0012 & pSB3K3-BBa_E0240

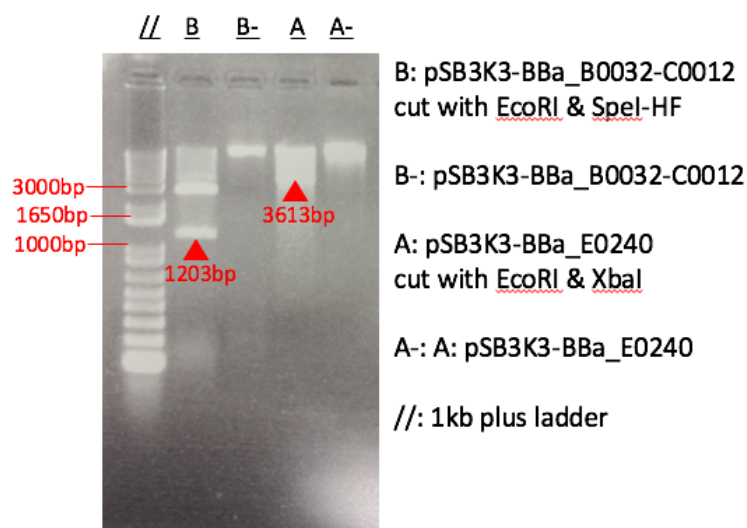


Figure . Restriction Digestion of pSB3K3-BBa_B0032-C0012 & pSB3K3-BBa_E0240

Gel Electrophoresis was ran at 1% agarose concentration at V/cm, in-gel stained by Midori Green. The Colony PCR products were compared to 8µl of 1kb plus DNA ladder.

Restriction Digestion of pSB3K3-BBa_B0032-C0012 and pSB3K3-BBa_E0240

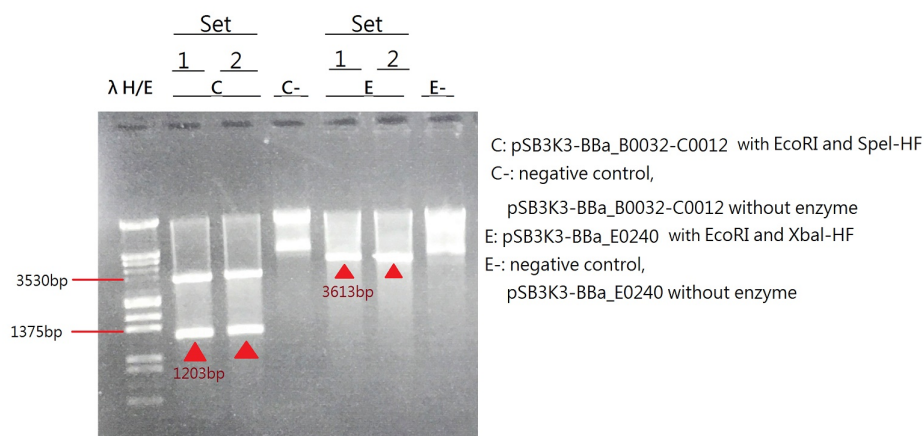


Figure . Restriction Digestion of pSB3K3-BBa_B0032-C0012 and pSB3K3-BBa_E0240

Gel electrophoresis was carried at 1% agarose concentration at 130V, in-gel stained by Midori Green. The plasmid samples underwent restriction digestion were compared to 6µl Lambda DNA/EcoRI+HindIII ladder.

11.Colony PCR of pSB3K3-BBa_B0032-C0012-E0240 with VF2 and VR primers

Colony PCR of pSB3K3-BBa_B0032-C0012-E0240

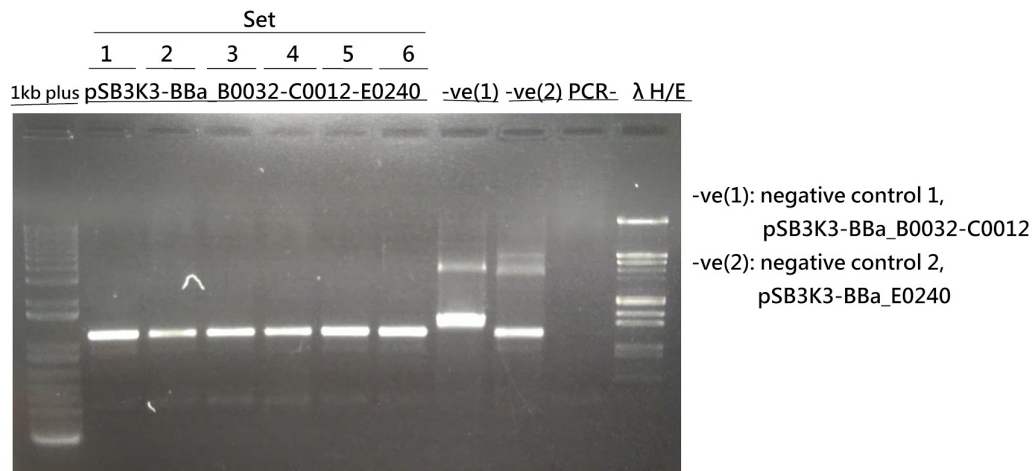


Figure . Colony PCR of pSB3K3-BBa_B0032-C0012-E0240

Gel electrophoresis was carried at 1% agarose concentration at 130V, in-gel stained by Midori Green. The DNA samples underwent Colony PCR were compared to 6 µl 1kb plus DNA ladder and 6µl Lambda DNA/EcoRI+HindIII.

Week 6 (11/07 - 15/07)

1. Restriction Digestion

- pSB3K3-BBa_J23101-B0032-C0040-B1006 (EcoRI, SpeI-HF)
- pSB3K3-BBa_R0040-E0240 (EcoRI, XbaI)

2. Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240 with VF2 and VR primers

Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240

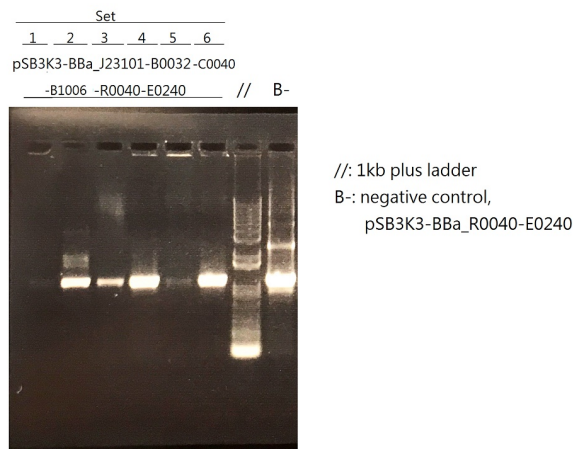


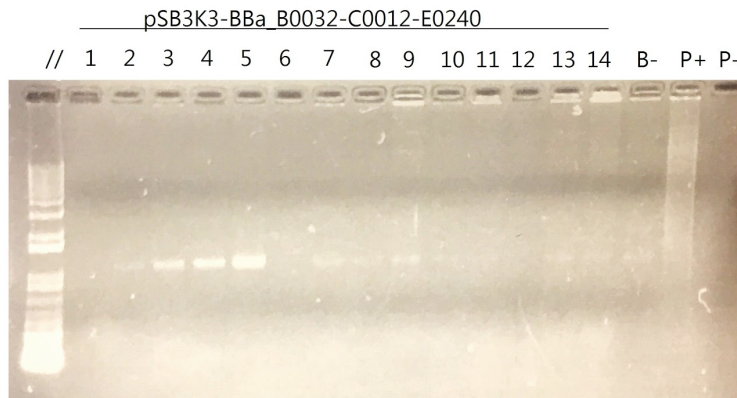
Figure . Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240

Gel electrophoresis was carried at 1% agarose concentration, at 130V, in-gel stained by Midori Green. The DNA samples underwent Colony PCR were compared to 6µl 1kb plus ladder.

Week 7 (18/07 - 21/07)

1. Colony PCR of pSB3K3-BBa_B0032-C0012-E0240 with VF2 and VR primers

Colony PCR of pSB3K3-BBa_B0032-C0012-E0240



B-: pSB3K3-BBa_E0240
P+: pSB3K3-BBa_I0500-E0240-
R0020-B0032-C0012-B0015-
K516032
P-: without template

Figure . Colony PCR of pSB3K3-BBa_B0032-C0012-E0240
Gel electrophoresis was carried at 1% agarose concentration, at 130V, in-gel stained by Midori Green. The plasmid samples underwent Colony PCR were compared to 6µl 1kb plus DNA ladder.

Colony PCR of pSB3K3-BBa_B0032-C0012-E0240



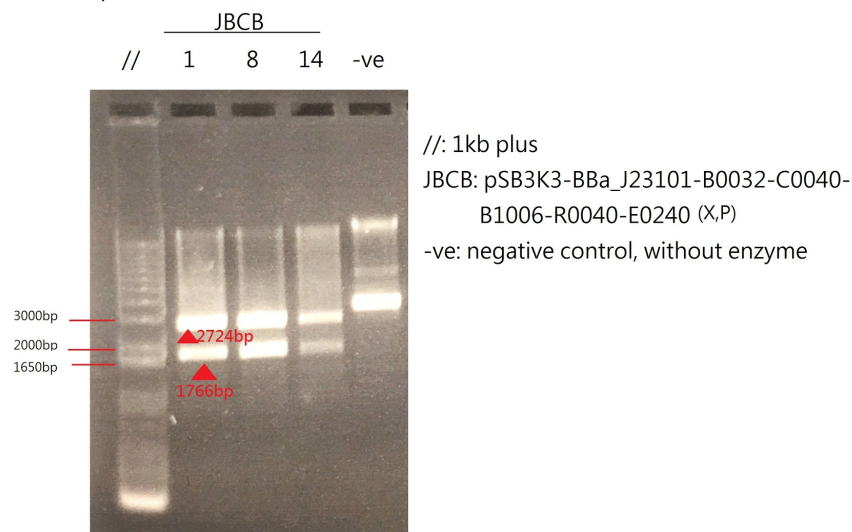
B-: pSB3K3-BBa_E0240
P+: pSB3K3-BBa_I0500-E0240-
R0020-B0032-C0012-B0015-
K516032
P-: without template

Figure . Colony PCR of pSB3K3-BBa_B0032-C0012-E0240
Gel electrophoresis was carried at 1% agarose concentration, at 130V, in-gel stained by Midori Green. The plasmid samples underwent Colony PCR were compared to 6µl 1kb plus DNA ladder.

2. Restriction digestion of pSB3K3-BBa_B0032-C0012-E0240

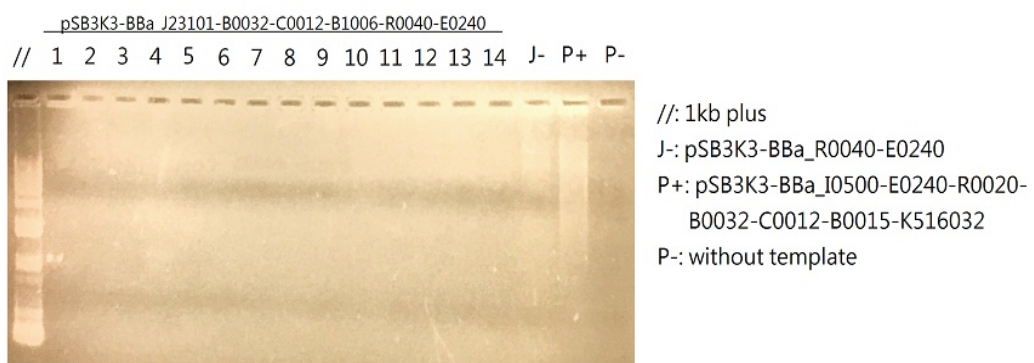
3. Restriction Check of pSB3K3-Bba_J23101-B0032-C0040-B1006-R0040-E0240

Restriction Check for pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240



4. Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240 with VF2 and VR primers

Colony PCR of pSB3K3-BBa_J23101-B0032-C0012-B1006-R0040-E0240



Colony PCR of pSB3K3-BBa_J23101-B0032-C0012-B1006-R0040-E0240

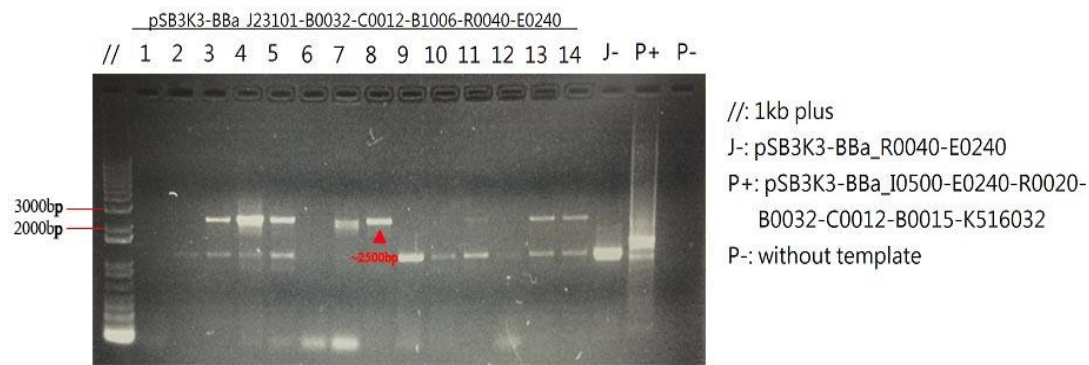


Figure. Colony PCR of pSB3K3-BBa_J23101-B0032-C0012-B1006-R0040-E0240
Gel electrophoresis was carried out at 1% agarose concentration, at 130V, in-gel stained by Midori Green. The plasmid samples underwent Colony PCR were compared to 6µl 1kb plus DNA ladder

5. Restriction Digestion of:

- pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240
- pSB3K3-BBa_B0032-C0012-E0240

Restriction digestion for pSB3K3-BBa_B0032-C0012-E0240 and
pSB3K3-BBa_J23101-B0032-C0012-B1006-R0040-E0240

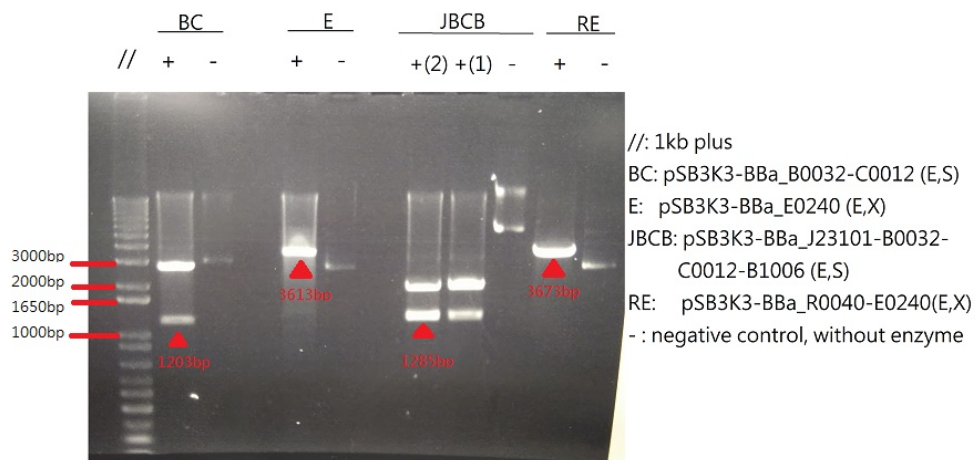


Figure . Restriction digestion for pSB3K3-BBa_B0032-C0012-E0240
and pSB3K3-BBa_J23101-B0032-C0012-B1006-R0040-E0240
Gel electrophoresis was carried at 1% agarose concentration, at 130V, in-gel stained by Midori Green. The plasmid samples underwent Restriction digestion was compared to 6µl 1kb plus DNA ladder.

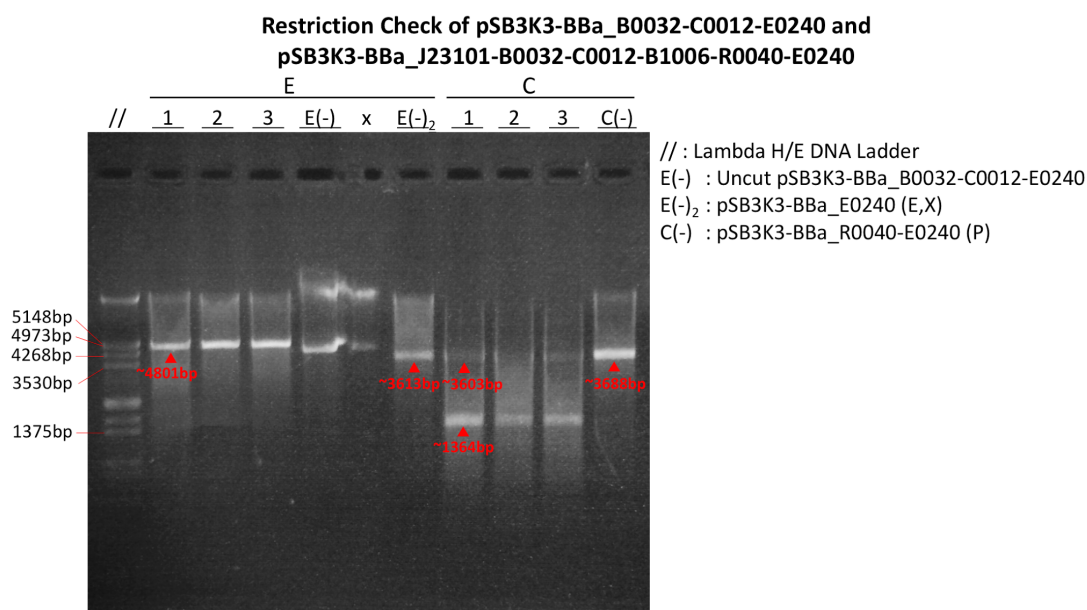


Figure . Restriction Check of Second Part of Construct E and Construct C
Gel electrophoresis was carried at 1% agarose concentration, at 130V, in-gel-stained by Midori Green.
The plasmid samples underwent restriction digestion (1hr30min, 37°C) were compared to 6μL
Lambda H/E DNA Ladder

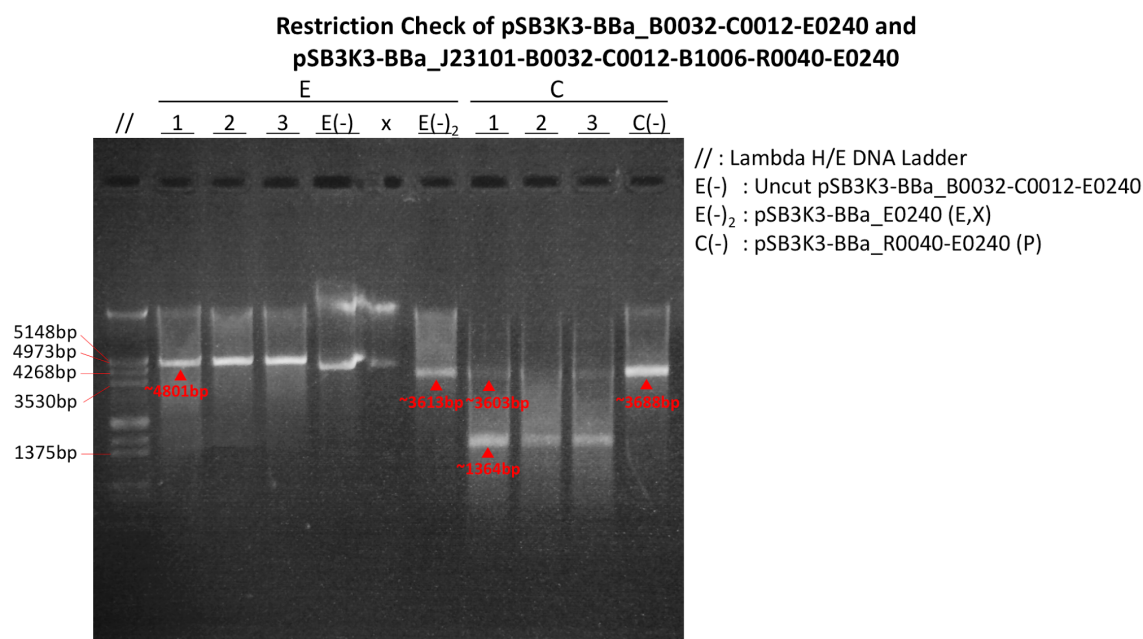


Figure . Restriction Check of Second Part of Construct E and Construct C
Gel electrophoresis was carried at 1% agarose concentration, at 130V, in-gel-stained by Midori Green.
The plasmid samples underwent restriction digestion (1hr30min, 37°C) were compared to 6μL
Lambda H/E DNA Ladder

Week 8 (25/07 - 03/08)

1. Phusion PCR of BBa_B0032-*phIF* with B0032-*phIF* FW and B0032-*phIF* RV primers

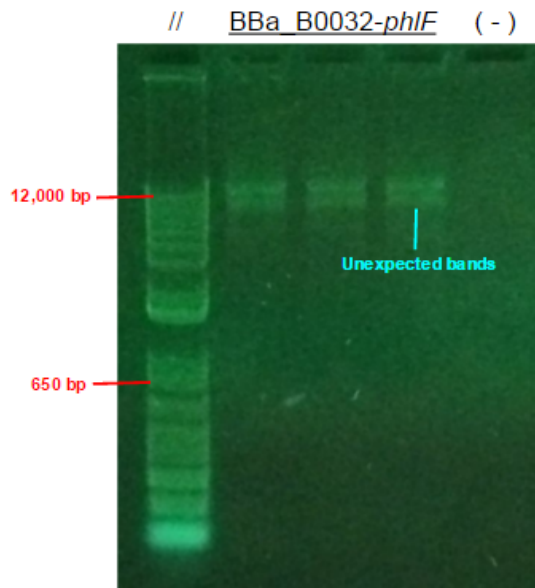


Figure 1. Phusion PCR of BBa_B0032-phIF.

Gel electrophoresis was carried out with 1% agarose gel concentration at 130V, stained by Midori Green. The DNA sample undergone Phusion PCR was compared to 6 μ l of 1kb plus DNA ladder.

(-): Negative control (no DNA template)

Expected band size: 665 bp

Conclusion:
Problem in DNA template

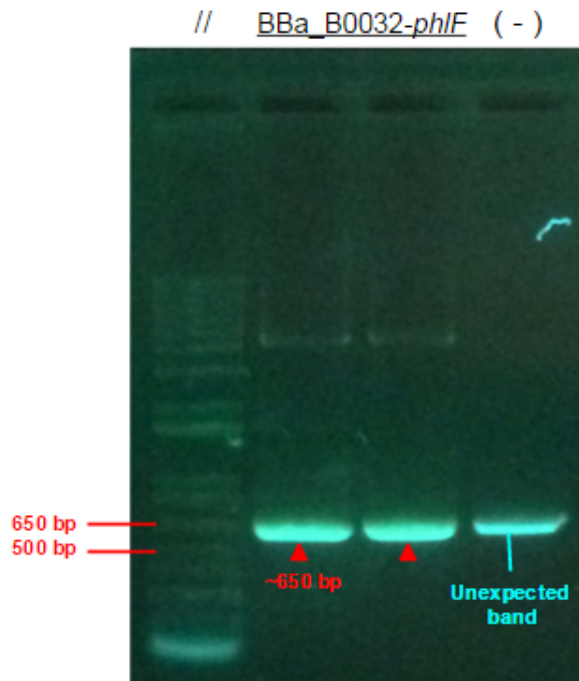


Figure 2. Phusion PCR of BBa_B0032-phIF

Gel electrophoresis was carried out with 1% agarose gel concentration at 130V, stained by Midori Green. The DNA sample undergone Phusion PCR was compared to 6 μ l of 1kb plus DNA ladder.

(-): Negative control (no DNA template)

Expected band size: 665 bp

Conclusion:
Possible contamination

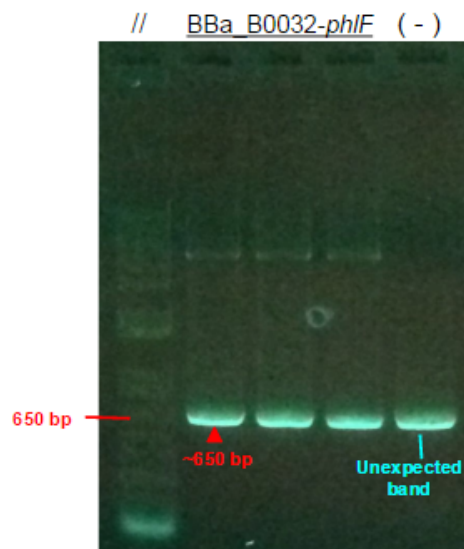


Figure 4. Phusion PCR of BBa_B0032-*phlF*

Gel electrophoresis was carried out with 1% agarose gel concentration at 130V, stained by Midori Green. The DNA sample undergone Phusion PCR was compared to 6 μ l of 1kb plus DNA ladder.

(-): Negative control (no DNA template)

Expected band size: 665 bp

2. Restriction Digestion of J61002-BBa_J23101 and BBa_B0032-*phlF*

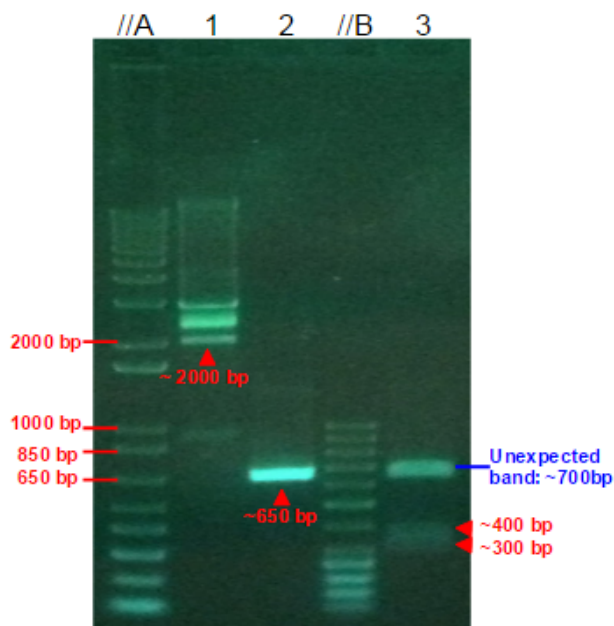


Figure 3. Restriction Digestion of J61002-BBa_J23101 & BBa_B0032-*phlF*

Gel electrophoresis was carried out with 1% agarose gel concentration at 130V, stained by Midori Green. The digested products were compared to 6 μ l of 1kb plus DNA ladder (Left) and 50 bp ladder (Right).

//A: 1kb plus DNA ladder

1 : J61002-BBa_J23101 (Cut at X, P)

2 : BBa_B0032-*phlF* (Cut at X, P)

//B: 50 bp ladder

3 : Restriction Check of BBa_B0032-*phlF* (Cut with XmnI)

Expected band sizes:

1: 2053 bp

2: 648 bp

3: ~300 bp and ~400 bp

Conclusion:

Only pSB1A2 was extracted

3. Restriction Digestion of J61002-BBa_J23101 and BBa_B0032-*phlF*

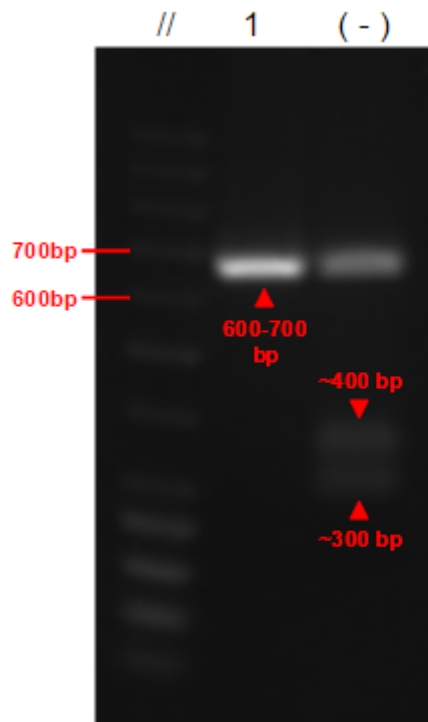


Figure 5. Restriction Digestion of J61002-BBa_J23101 & BBa_B0032-*phlF*.

Gel electrophoresis was carried out with 1% agarose gel concentration at 120V for 1 hour and 30 minutes, stained by Midori Green. The digested products were compared to 6 μ l of 50 bp DNA ladder.

// : 50 bp DNA ladder

1 : BBa_B0032-*phlF* (Cut at X, P)

(-): Restriction check of PCR Negative Control Band (Cut with *XmnI*)

Expected band size: 648 bp

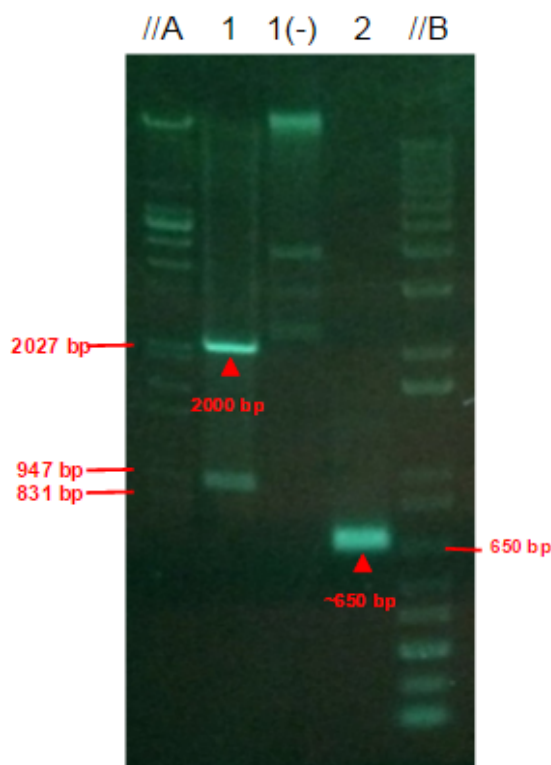


Figure 6. Restriction Digestion of J61002-BBa_J23101 & BBa_B0032-*phlF*.

Gel electrophoresis was carried out with 1% agarose gel concentration at 120V for 1 hour and 30 minutes, stained by Midori Green. The digested products were compared to 6 μ l of Lambda/EcoRI+HindIII and 1 Kb Plus DNA ladder.

//A : Lambda/EcoRI+HindIII DNA Ladder

1 : J61002-BBa_J23101 (Cut at X, P)

1(-): J61002-BBa_J23101 (Uncut)

2 : BBa_B0032-*phlF* (Cut at X, P)

//B : 1 Kb plus DNA Ladder

Expected band sizes:

1: 2053 bp

2: 648 bp

Conclusion:

Desired parts obtained and purified

4. Ligation of pSB1A2 backbone with BBa_B0032-*phlF*

5. Transformation of pSB1A2-BBa_B0032-*phlF*

6. Checking of Digested and Purified pSB1A2 (X, P) Backbone



Figure 7. Checking of Digested and Purified pSB1A2 (X, P) Backbone.

Gel electrophoresis was carried out with 1% agarose gel concentration at 130V for ~45 minutes, stained by Midori Green. The band was compared to 6 μ l 1Kb Plus Ladder.

1: pSB1A2 (X, P)
//: 1 Kb plus Ladder

Expected band size: 2053 bp

Conclusion:
pSB1A2 backbone is present in purified product.

7. Restriction Check of pSB1A2-BBa_B0032-phIF

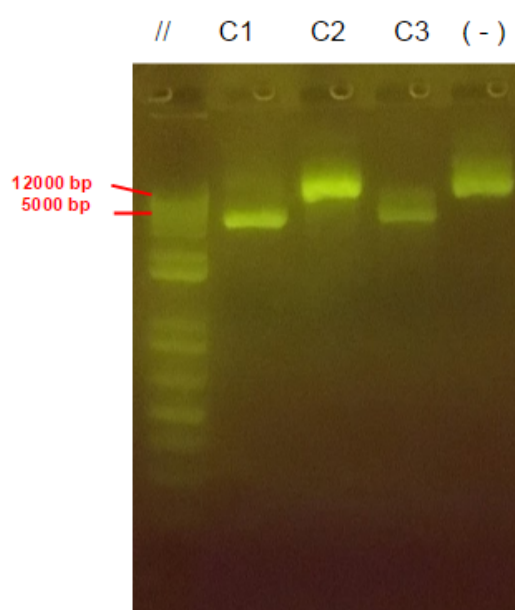


Figure 8. Restriction Check of pSB1A2-BBa_B0032-phIF with PvuII enzyme.

Gel electrophoresis was carried out with 1% agarose gel concentration at 130V for 30 minutes, stained by Midori Green. The digested products were compared to 6 μ l of 1 Kb Plus DNA ladder.

// : 1 Kb plus DNA Ladder
C1,2,3 : pSB1A2-BBa_B0032-phIF
(-) : J61002-BBa_J23101

Expected band sizes:
C1,2,3: 2524 bp, 153 bp, 24 bp
(-) : 2983 bp

Conclusion:
Possibly problem with picked colony or miniprep

Week 9 (04/08 - 05/07)

1. Characterisation of:

- pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240 (Construct B)
- pSB3K3-BBa_J23101-B0032-C0012-B1006-R0040-E0240 (Construct C)

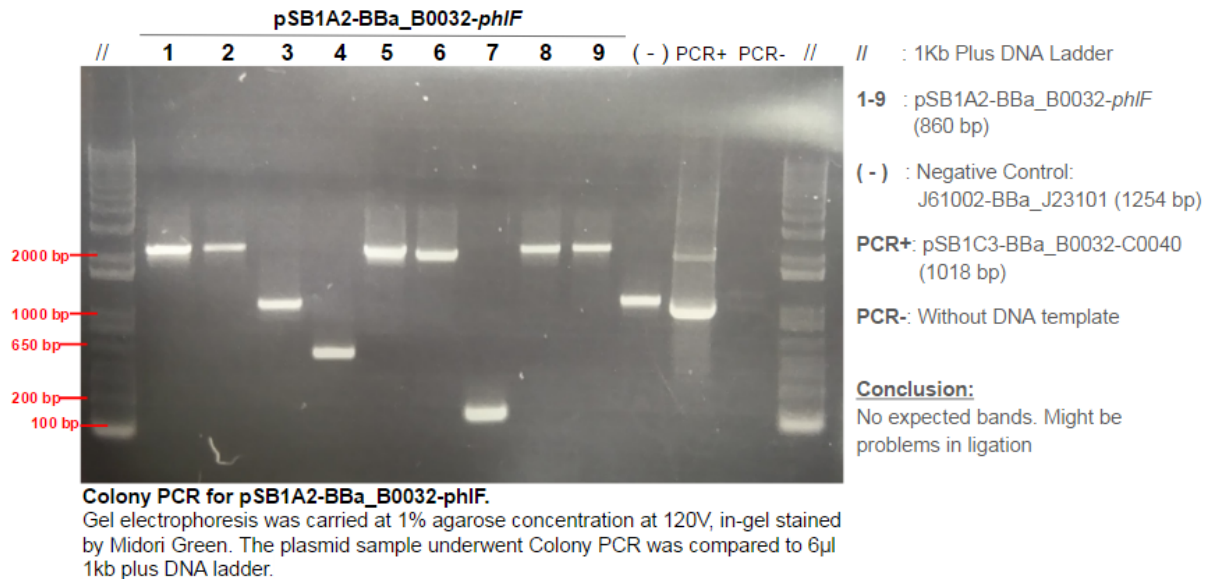
Week 10 (08/08 - 12/08)

1. Characterisation of:

- pSB3K3-BBa_R0040-E0240 (Construct A)
- pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240 (Construct B)
- pSB3K3-BBa_J23101-B0032-C0012-B1006-R0040-E0240 (Construct C)

2. Ligation of pSB1A2 with B0032-phIF

3. Colony PCR of pSB1A2-BBa_B0032-phIF with VF2 and VR primers



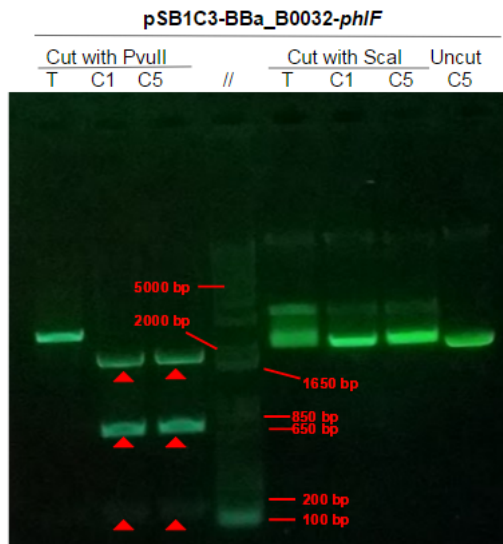
Week 10 (15/08 - 19/08)

1. Characterisation

- pSB3K3-BBa_R0040-E0240 (Construct A)
- pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240 (Construct B)

2. Ligation of pSB1C3 with BBa_B0032-phIF

3. Restriction Check of pSB1C3-BBa_B0032-phIF



Restriction Check for pSB1C3-BBa_B0032-phIF.

Gel electrophoresis was carried at 1% agarose concentration at 130V, in-gel stained by Midori Green. The plasmid sample underwent Colony PCR was compared to 6µl 1kb plus DNA ladder.

Legends

T = Template DNA

pSB1A2-BBa_B0034-phIF

C1, C5 = Candidates 1 and 2 of

pSB1C3-BBa_B0032-phIF

Expected bands

C1, C5 = PvuII: 1780 bp, 735 bp and 153 bp

Scal: 1769 bp and 896 bp

T = PvuII: 1735 bp, 736 bp and 153 bp

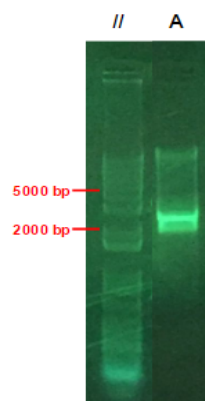
Scal: 2648 bp

Week 11 (22/08 - 26/08)

1. Functional Assay of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240 (Construct B)
2. Characterization of:
 - pSB3K3-BBa_R0040-E0240 (Construct A)
 - pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240 (Construct B)
 - pSB3K3-BBa_J23101-B0032-C0012-B1006-R0040-E0240 (Construct C)

Week 12 (30/08 - 02/09)

1. Characterisation of:
 - pSB3K3-BBa_R0040-E0240 (Construct A)
 - pSB3K3-BBa_J23101-B0032-C0012-B1006-R0040-E0240 (Construct C)
2. Restriction Digestion of pSB1C3-BBa_B0032-phIF



30 August

Restriction Digestion of pSB1C3-BBa_B0032-phIF.

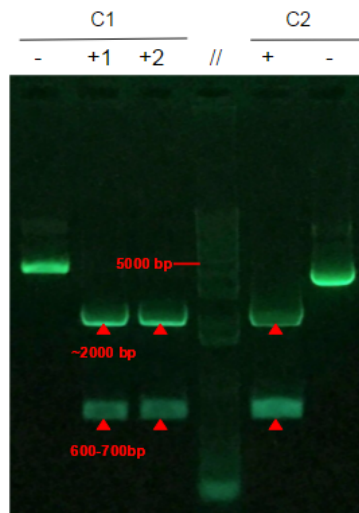
Gel electrophoresis was carried at 1% agarose concentration at 130V, in-gel stained by Midori Green. The plasmid sample underwent Colony PCR was compared to 6µl 1kb plus DNA ladder.

Legends

//: 1 Kb Plus DNA Ladder

A: pSB1C3-BBa_B0032-phIF (XbaI & PstI)

Expected band sizes: 2044bp & 648bp



2 September

Restriction Digestion of pSB1C3-BBa_B0032-phIF.

Gel electrophoresis was carried at 1% agarose concentration at 130V, in-gel stained by Midori Green. The plasmid sample underwent Colony PCR was compared to 6µl 1kb plus DNA ladder.

Legends

// : 1 Kb Plus DNA Ladder

C1,2: Candidates 1 and 2 of pSB1C3-BBa_B0032-phIF (XbaI & PstI)

+ : Digested sample with XbaI and PstI-HF enzymes

- : Undigested sample

Expected band sizes: 2044bp & 648bp

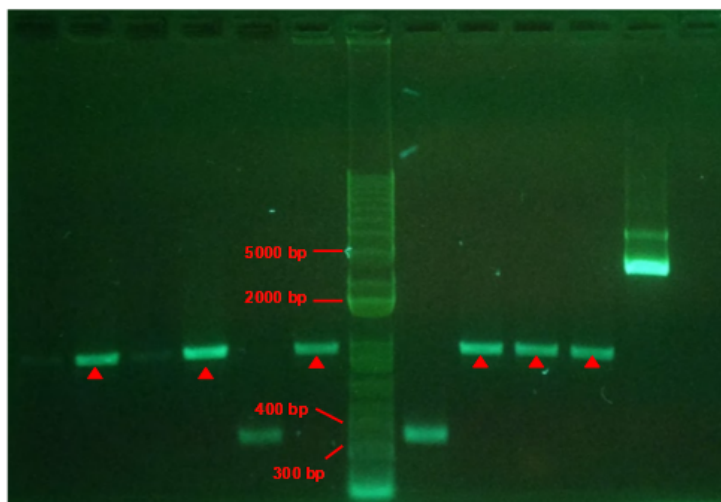
Week 13 (05/09 - 09/09)

1. Characterisation of:

- pSB3K3-BBa_R0040-E0240 (Construct A)
- pSB3K3-BBa_J23101-B0032-C0012-B1006-R0040-E0240 (Construct C)

2. Colony PCR of pSB1C3-BBa_R0040-B0032-phIF with VF2 and VR primers

C1 C2 C3 C4 C5 C6 // C7 C8 C9 C10 (+) (-)



Colony PCR of

pSB1C3-BBa_R0040-B0032-phIF.

Gel electrophoresis was carried at 1% agarose concentration at 130V, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

Legends

// : 1 Kb Plus DNA Ladder

C1-10 : Candidates 1 to 10

pSB1C3-BBa_R0040-B0032-phIF

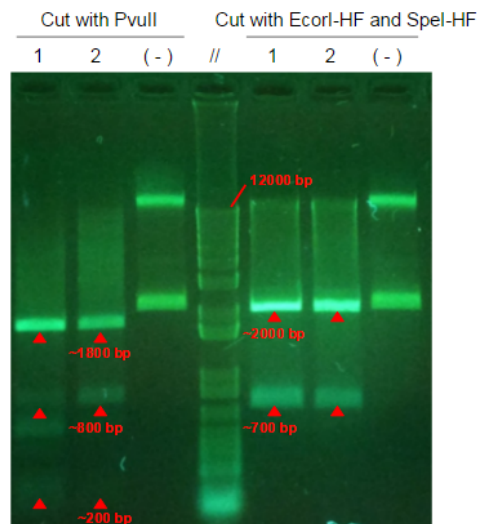
(+) : pSB3K3-BBa_R0040-E0240

(-) : No DNA template

Expected band size: 998 bp

(C5 and C7 are suspected to be the backbone; size: 368bp)

3. Restriction Check of pSB1C3-BBa_R0040-B0032-phIF



Restriction Check of pSB1C3-BBa_R0040-B0032-phIF.
Gel electrophoresis was carried at 1% agarose concentration at 130V, in-gel stained by Midori Green. The plasmid sample was compared to 6 μ l 1 Kb plus DNA ladder.

Legends

// : 1 Kb Plus DNA Ladder

1,2 : Candidates 8 and 9 of pSB1C3-BBa_R0040-B0032-phIF respectively

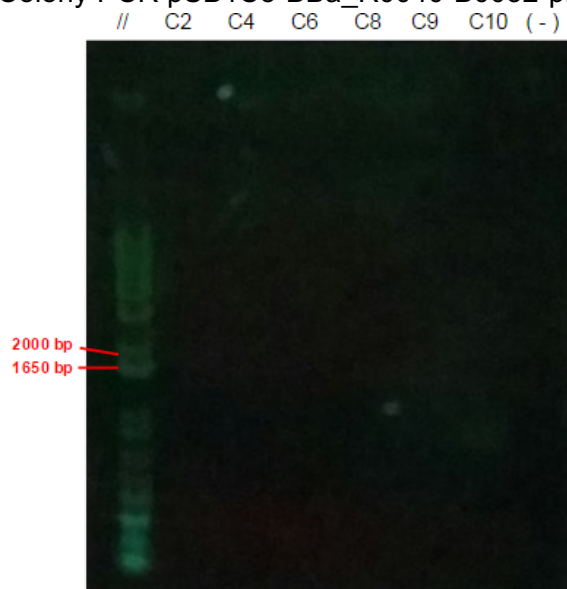
(-) : Undigested Candidate 1

Expected band sizes:

PvuII: 24bp, 153bp, 797bp, 1780bp

EcoRI-HF and SpeI-HF: 707bp, 2047bp

4. Colony PCR pSB1C3-BBa_R0040-B0032-phIF with sptet(-35-10) and VR primers



Colony PCR of pSB1C3-BBa_R0040-B0032-phIF with specific primer.

Gel electrophoresis was carried at 1% agarose concentration at 130V, in-gel stained by Midori Green. The plasmid samples were compared to 6 μ l 1 Kb plus DNA ladder.

Legends

// : 1 Kb Plus DNA Ladder

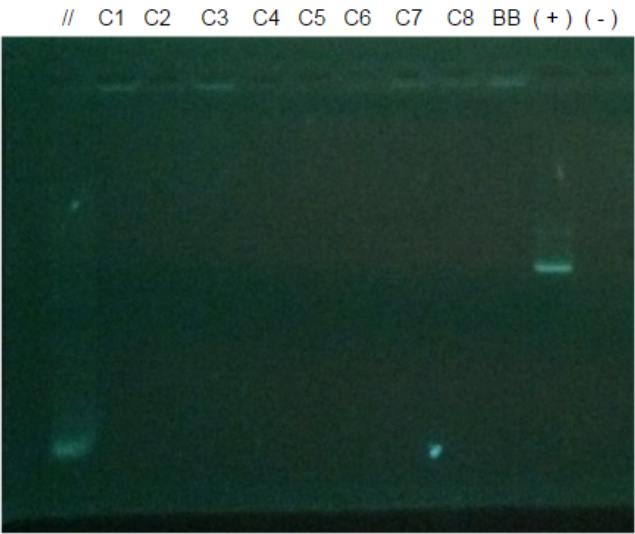
C1-10 : Candidates 1 to 2 of pSB1C3-BBa_R0040-B0032-phIF

(-) : No template DNA

Expected band size: 860bp

Week 14 (12/09 - 16/09)

1. Colony PCR of pSB1C3-BBa_R0040-B0032-*phlF* with Sptet(-35-10) and VR primers

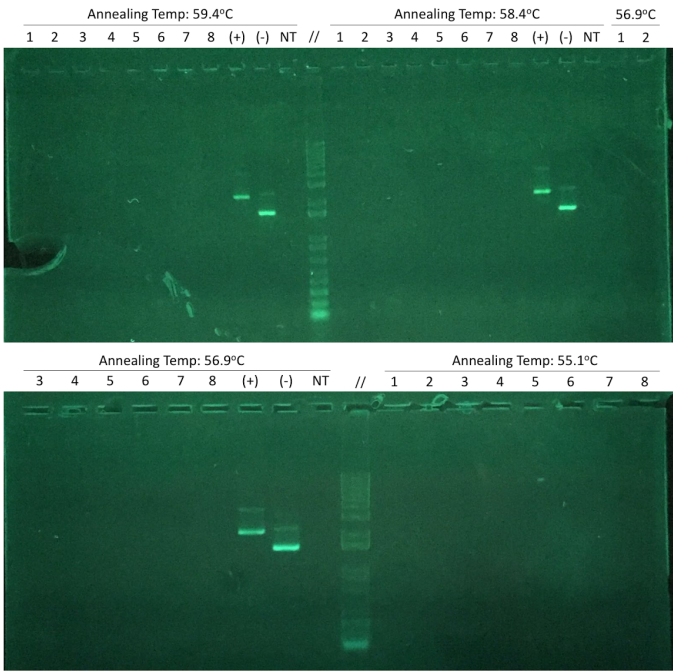


Colony PCR of pSB1C3-BBa_R0040-B0032-*phlF*.
Gel electrophoresis was carried at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

Legends
// : 1 Kb Plus DNA Ladder
C1 - 8 : Candidates 1 to 8
pSB1C3-BBa_R0040-B0032-*phlF*
BB : pSB1C3-BBa_R0040
(+) : pSB3K3-BBa_R0040-E0240
(-) : No DNA template

Expected band size: 998 bp
(C5 and C7 are suspected to be the backbone; size: 368bp)

2.Gradient Colony PCR of pSB1C3-BBa_R0040-B0032-*phlF* with Sptet(-35-10) and VR primers

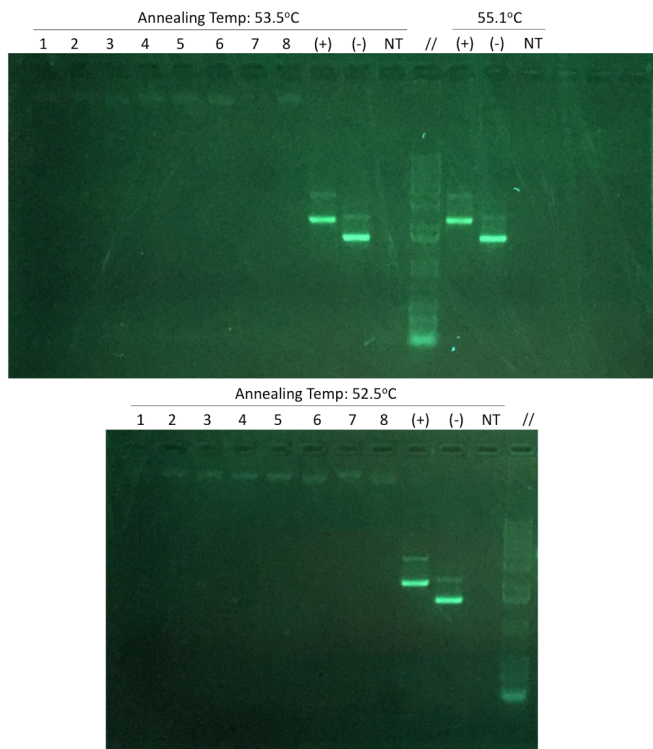


Gradient PCR of pSB1C3-BBa_R0040-B0032-*phlF* with sptet(-35-10) & VR

Colony PCR of pSB1C3-BBa_R0040-B0032-*phlF*
with annealing temperature gradient, amplified by using sptet(-35-10) and VR primers. Gel electrophoresis was carried at 1% agarose concentration at 130V, in-gel stained by Midori Green. The plasmid samples were compared to 1kb plus DNA ladder.

Legends
// : 1kb plus DNA ladder
1-8 : 8 different candidates of pSB1C3-BBa_R0040-*phlF*
(+) : pSB1C3-BBa_R0040-E0240
(-) : pSB1C3-BBa_R0040
NT : No DNA template

Expected band size: 860bp



Gradient PCR of pSB1C3-BBa_R0040-B0032-*phIF* with sptet(-35-10) & VR

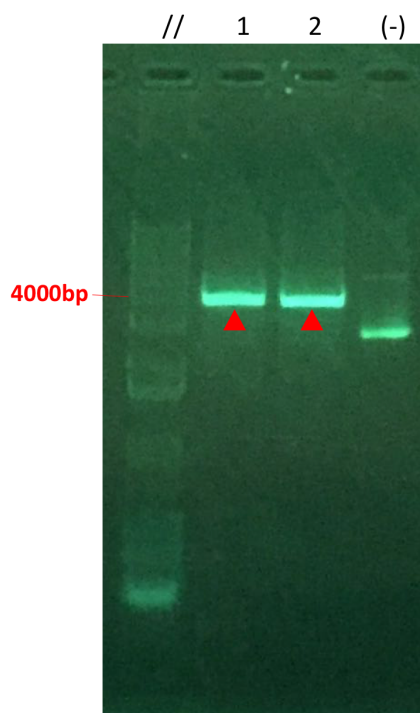
Colony PCR of pSB1C3-BBa_R0040-B0032-*phIF* with annealing temperature gradient, amplified by using sptet(-35-10) and VR primers. Gel electrophoresis was carried at 1% agarose concentration at 130V, in-gel stained by Midori Green. The plasmid samples were compared to 1kb plus DNA ladder.

Legends

// : 1kb plus DNA ladder
 1-8 : 8 different candidates of pSB1C3-BBa_R0040-*phIF*
 (+) : pSB1C3-BBa_R0040-E0240
 (-) : pSB1C3-BBa_R0040
 NT : No DNA template

Expected band size: 860bp

3. Restriction Digestion of pSB3K3-BBa_B0032-*phIF*-B0032-C0012



Restriction Digestion of pSB3K3-BBa_B0032-C0012 with EcorI-HF and XbaI.

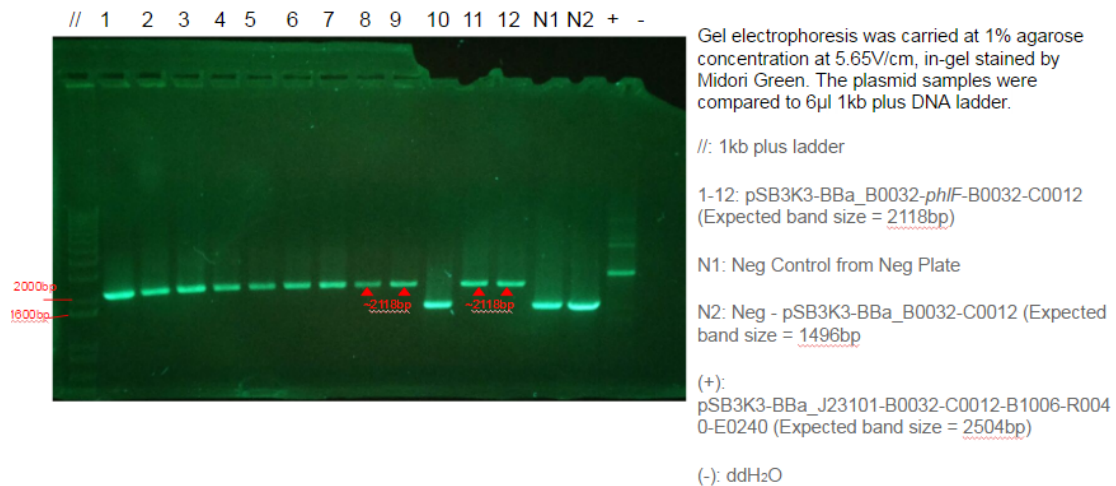
Gel electrophoresis was carried at 1% agarose concentration at 130V, in-gel stained by Midori Green. The plasmid samples were compared to 1kb plus DNA ladder.

Legends

// : 1kb plus DNA ladder
 1&2 : 2 samples of pSB1C3-BBa_B0032-C0012
 (-) : Uncut plasmid sample
 NT : No DNA template

Expected band size: 3907bp

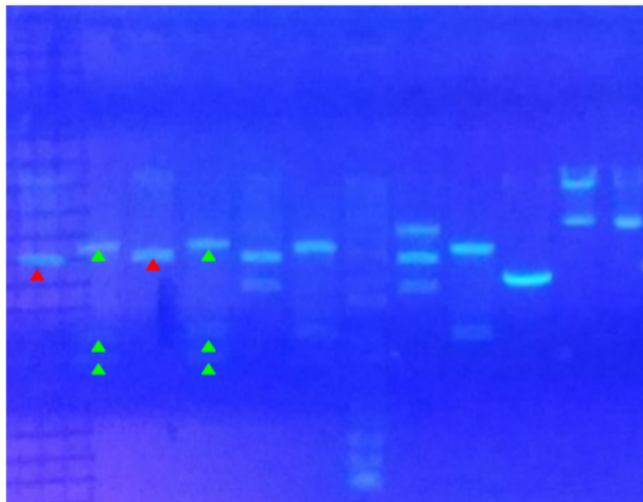
4. Colony PCR of pSB3K3-BBa_B0032-*phIF*-B0032-C0012 with VF2 and VR primers



Week 15 (19/09 - 23/09)

1. Transformation of pSB3K3-BBa_R0040-B0032-*phIF*-B0032-C0012-E0240
2. Restriction Check of pSB3K3-BBa_R0040-B0032-*phIF*-B0032-C0012-E0240 (Construct E)

C2		C5		
E,S	PvuII	E,S	PvuII	// [other cons] [other construct] C2- C5-



Gel electrophoresis was carried at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

Legends

// : 1 Kb Plus DNA Ladder

C2&5 : Candidates 2 and 5

C2-&5- : Uncut candidates 2 and 5

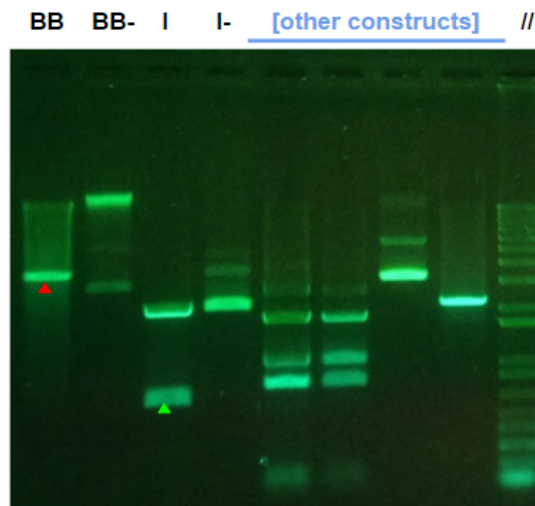
Expected band sizes

Cut at E,S : 2771 & 2727 bp ▲

Cut with PvuII: 3363, 1062, 803, 153, 93, 24 bp ▲

3. Restriction Digestion of:

- pSB3K3-BBa_R0040-E0240
- pSB1C3-BBa_J23101-B0032-*phIF*-B1006



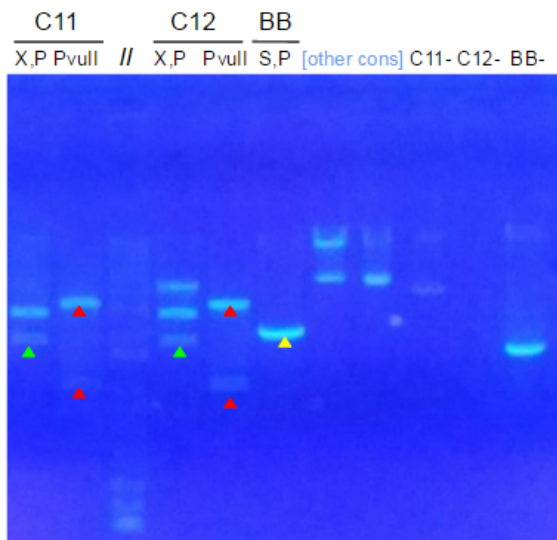
Gel electrophoresis was carried at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

Legends

// : 1 Kb Plus DNA Ladder
 BB : Backbone, pSB3K3-BBa_R0040-E0240
 cut at E, X (3673 bp) ▲
 BB- : Uncut backbone
 I : Insert, pSB1C3-BBa_J23101-B0032-phIF-B1006
 cut at E, S (735, 2047 bp) ▲
 I- : Uncut insert

4. Restriction Check of:

- pSB3K3-BBa_B0032-C0012-B0032-phIF
- pSB1C3-BBa_R0040



Gel electrophoresis was carried at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

Legends

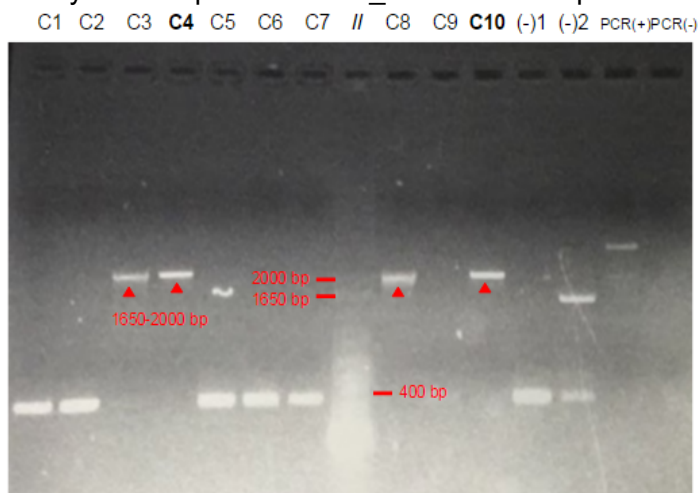
// : 1 Kb Plus DNA Ladder
 C11&12 : Insert, pSB3K3-BBa_B0032-C0012-B0032-phIF
 BB : Backbone, pSB1C3-BBa_R0040
 (-) : Uncut sample

Expected band sizes

Insert cut with pvull : 3220, 1070, 153, 93, 24 bp ▲
 Insert cut at X, P : 2724 and 1836 bp ▲
 Backbone cut at S, P : 2106bp ▲

Insert-C11 was used for ligation with Backbone, and the ligated product was transformed

5. Colony PCR of pSB1C3-BBa_R0040-B0032-phIF-B0032-C0012 with VF2 and VR primers



Gel electrophoresis was carried at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

Legends

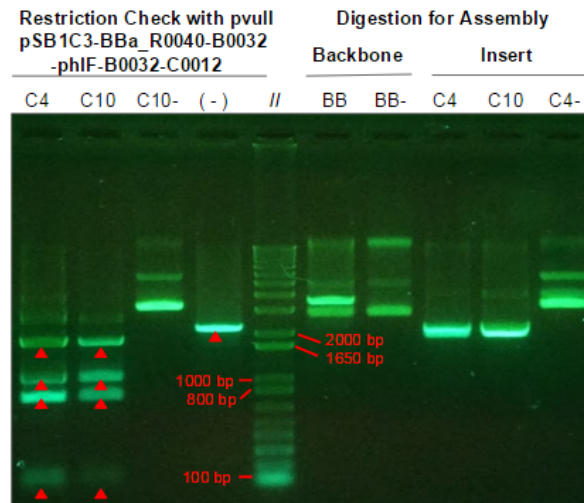
// : 1 Kb Plus DNA Ladder
 C1 - 10 : Candidates 1 to 10
 pSB1C3-BBa_R0040-B0032-phIF
 -B0032-C0012
 (-)1 : Sample from negative plate.
 (-)2 : pSB1C3-BBa_R0040
 PCR(+) : pSB3K3-BBa_B0032-phIF-B0032-C0012
 PCR(-) : No DNA template

Expected band size: 1885 bp

(Other bands of the candidates are suspected to be the backbone; size: 368)

6. Restriction Check of:

- pSB1C3-BBa_R0040-B0032-phIF-B0032-C0012
- pSB3K3-BBa_K1899000



Gel electrophoresis was carried at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

Legends

// : 1 Kb Plus DNA Ladder

Restriction Check with *pvuII*

C4&10 : Candidates 4 and 10 of pSB1C3-BBa_R0040-B0032-phIF-B0032-C0012 (24, 93, 153, 797, 1062, 1805 bp)

C10- : Uncut Candidate 10

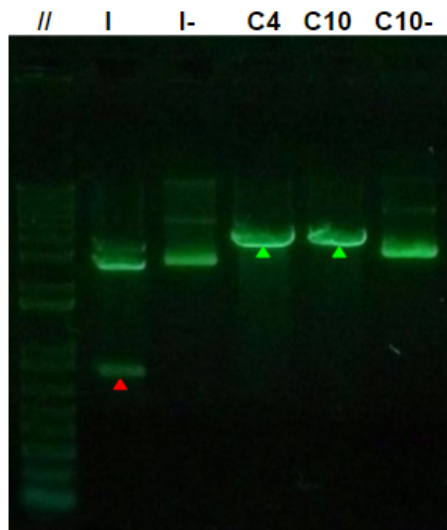
(-) : pSB1C3-BBa_R0040 (2124 bp)

Digestion for Assembly

BB : Backbone, pSB3K3-BBa_K1899000 cut with E, X (15, 3506 bp)

BB- : Uncut backbone

C4&10 : Insert, pSB1C3-BBa_R0040-B0032-phIF-B0032-C0012 cut with E, S (2047 and 1887 bp)



Gel electrophoresis was carried at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

Legends

// : 1 Kb Plus DNA Ladder

I : Insert, pSB3K3-BBa_K1899000 cut with X, P (2724, 797 bp) ▲

I- : Uncut insert

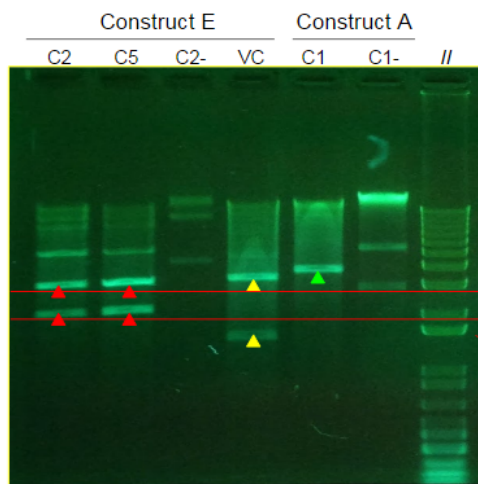
C4&10 : Backbone, Candidates 4 and 10 of pSB1C3-BBa_R0040-B0032-phIF-B0032-C0012 cut with S, P (18 and 3916 bp)

C10- : Uncut backbone, Candidate 10

Week 16 (26/09 - 30/09)

1. Restriction Digestion of:

- pSB3K3-BBa_R0040-E0240 (Construct A)
- pSB3K3-BBa_R0040-B0032-phIF-B0032-C0012-E0240 (Construct E)



Gel electrophoresis was carried at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

Legends

// : 1 Kb Plus DNA Ladder

Construct E

pSB3K3-BBa_R0040-B0032-*phlF*-B0032-C0012-E0240

(*pTet-phlF-LacI*-GFP)

C2&5 : Candidates 2 and 5, cut with *Styl* (3296, 2202 bp ▲)

VC : Vector Control - without GFP

pSB3K3-BBa_R0040-B0032-*phlF*-B0032-C0012

Cut with *Styl* (3296, 1512 bp ▲)

C2- : Uncut candidate 2

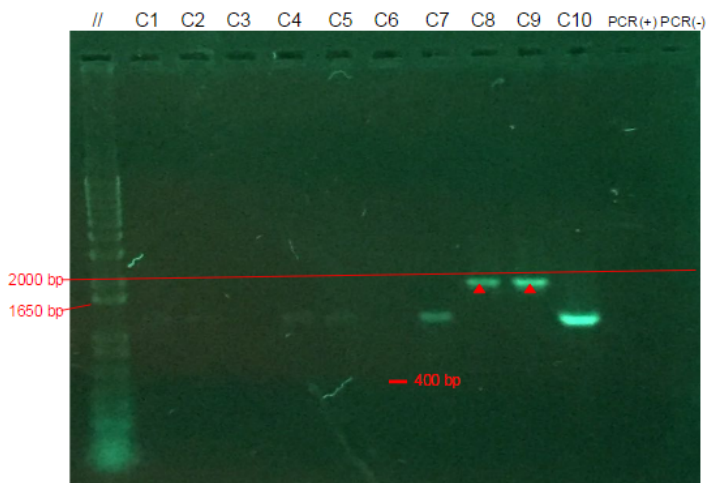
Construct A

pSB3K3-BBa_R0040-E0240 (*pTet*-GFP)

C1 : Candidate 1, cut at E and X (3673 bp ▲)

C1- : Uncut candidate 1

2. Colony PCR of pSB3K3-BBa_J23101-B0032-*phlF*-B1006-R0040-E0240 (Construct D) with VF2 and VR primers



Gel electrophoresis was carried at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

Legends

// : 1 Kb Plus DNA Ladder

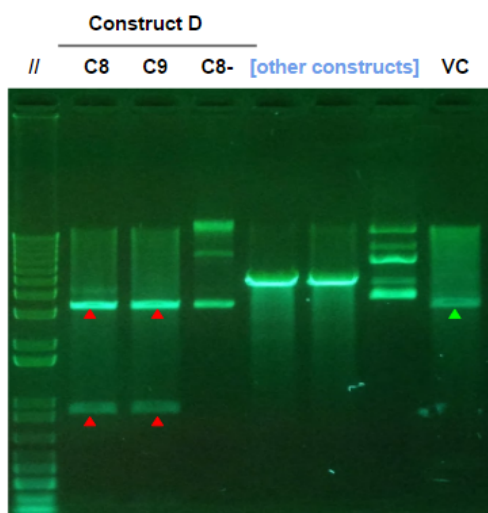
C1 - 10 : Candidates 1 to 10
pSB3K3-BBa_J23101-B0032-*phlF*-
B0032-C0012-R0040-E0240

PCR(+) : Construct E:
pSB3K3-BBa_R0040-B0032-
phlF-B0032-C0012-E0240 (3064 bp)

PCR(-) : No DNA template

Expected band size: 1974 bp

3. Restriction Check of pSB3K3-J23101-B0032-*phlF*-B1006-R0040-E0240 (Construct D)



Gel electrophoresis was carried at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

Legends

// : 1 Kb Plus DNA Ladder

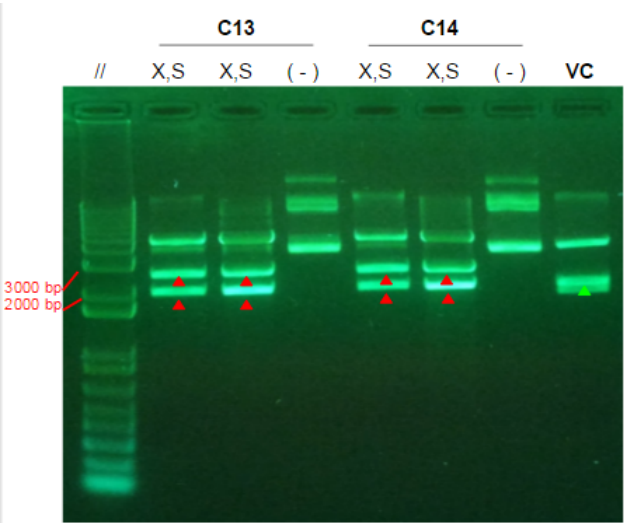
C8&9 : Candidates 8 and 9 of Construct D
Cut with *Styl* (3296, 949, 163 bp ▲)

C8- : Uncut Candidate 8

VC : Vector Control - Construct A
pSB3K3-BBa_R0040-E0240
Cut with *Styl* (3296, 392 bp ▲)

4. Restriction Check of pSB1C3-BBa_R0040-B0032-*phlF*-B0032-C0012-K1899000 for

Strand 1 of final construct



Gel electrophoresis was carried at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

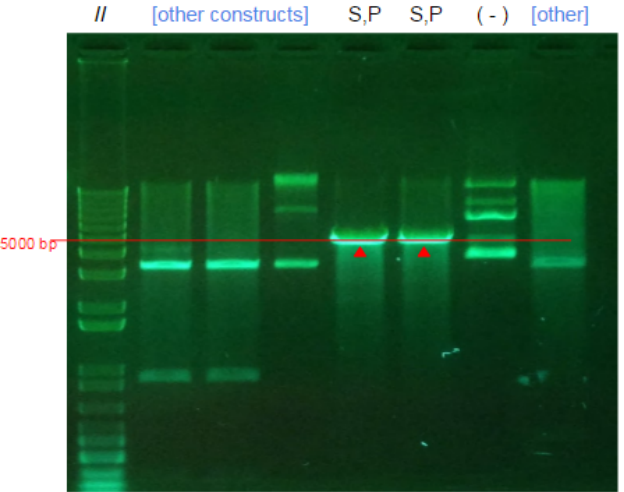
Legends

// : 1 Kb Plus DNA Ladder

C13&14 : Candidates 13&14 of Strand 1 of Final Construct
Cut at X,S (2651, 2062 bp ▲)

(-) : Uncut Sample

VC : Vector Control: without BFP
pSB1C3-BBa_R0040-B0032-phIF-B0032-C0012
Cut at X,S (2062, 1872 bp ▲)



Gel electrophoresis was carried at 1% agarose concentration at 5.65V/cm, in-gel stained by Midori Green. The plasmid samples were compared to 6µl 1kb plus DNA ladder.

Legends

// : 1 Kb Plus DNA Ladder

Expected Band Size: 4703 bp ▲